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A 02

INITIAL SUB-MISSION

A 03

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INTERNATIONAL ISOCYANATE INSTITUTE, INC.
201 Main Street, Suite 403 • La Crosse, WI 54601 • 608/796-0880 • FAX 608/796-0882

January 19, 2000

TSCA Document Processing Center (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

MR 31636

Attn: 8(e) Coordinator

RE: Polymeric diphenylmethane diisocyanate
9016-87-9

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2000 FEB -1 AM 11: 10

Dear Sir/Madam:

The following information is being submitted by the International Isocyanate Institute (III) on behalf of its members[1] pursuant to current guidance issued by EPA indicating EPA's interpretation of Section 8 (e) of the Toxic Substance Control Act. Neither III nor any member of III has made a determination as to whether a significant risk of injury to health or the environment is actually presented by the findings.

The enclosed report, III Ref 1136 ***"...meric MDI: 28 Day Inhalation Toxicity Study in Female Rats with Post-Exposure Observation ... ...s up to 30 Days,"*** by N.S. Rattray was recently issued by the III Scientific Office. This study helps to explain published information as Rattray concludes that the findings "are consistent with an exposure to a mildly irritant aerosol."

Sincerely,

M.J. Blankenship
Managing Director

Enclosure: Report

cc: J. Chapman
D. Gilbert
J. Jadlocki
J. Lyon
T. Landry
R. Robert
M. Spence

Contain NO CBI

[1] Members in the Americas Region: BASF Corporation, Bayer Corporation, Dow Chemical Company, Huntsman Polyurethanes and Lyondell.

A 04

III Project 151

III ref 11362

Polymeric MDI: 28 day inhalation toxicity study in female rats with post-exposure observation periods up to 30 days

N J Rattray
Central Toxicology Laboratory
Alderley Park
Macclesfield
Cheshire
UK

Number of pages: 90

1. SUMMARY

1.1 Study design

Groups of fifteen or twenty female Alpk:AP$_f$SD (Wistar-derived) rats were exposed nose-only to a target concentration of 0 (control), 1, 4 or 10mg/m^3 polymeric MDI for 6 hours per day, 5 days per week, over a 4 week period and terminated the day after the last exposure (main study). Additional groups of fifteen or twenty animals were similarly exposed and retained without treatment for a further 30 days (recovery phase).

Clinical observations and bodyweights were recorded weekly throughout the study. One week prior to scheduled termination selected animals for 'S' phase analysis were implanted subcutaneously with an osmotic minipump for delivery of bromodeoxyuridine (BrdU). At the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem*. Selected animals were taken for subsequent histopathology examination, lungs were weighed and specified tissues were submitted for light and electron microscopy and to provide an assessment of cell proliferation. In addition an attempt was made to analyse the particulate material that is a consistent observation in alveolar macrophages of MDI exposed rats. Lung lavage samples were taken from specified animals and examined for any changes in alveolar fluid compartment namely proteins, enzymes and phospholipids and associated free cell population.

1.2 Results

The test atmospheres generated of 1, 4 or 10mg/m^3 MDI (actual achieved concentrations 0.93, 3.88 or 10.3mg/m^3 MDI) were acceptable with regard to their general stability and physical characteristics.

Group	Total particulate concentration mg/m^3	Median size (MMAD) (µm)	GSD
2	0.93	1.14	2.47
3	3.88	1.61	1.95
4	10.3	1.09	1.68

There were no clinical changes or effects on bodyweight. The lung lavage samples showed a statistically significant increase in total cell count and alveolar macrophage count in animals exposed to 10.3mg/m^3 MDI on the main study, there was a less marked increase, which did not achieve statistical significance, in animals exposed to 3.88mg/m^3. There was a statistically significant increase in polymorphonuclear leucocytes and in lymphocyte/other cells in main study animals exposed to 3.88 or 10.3mg/m^3 MDI. The numbers of macrophages with distinct vacuoles, termed foamy macrophages, showed a large increase in main study animals treated with 10.3mg/m^3 MDI and a less marked increase in animals exposed to 3.88mg/m^3 MDI. In the lung lavage fluid, total protein and alkaline phosphatase activity were statistically significantly increased in main study animals exposed to 10.3mg/m^3 MDI. In the lung lavage fluid and in the cell pellet main study animals exposed to 10.3mg/m^3 MDI showed a statistically significant increase in both supernatant and intracellular levels of phosphatidylcholins, in addition main study animals exposed to 3.88mg/m^3 MDI showed a statistically significant increase in intracellular levels of phosphatidylcholins. Main study animals exposed to 10.3mg/m^3 showed an increase in lung weight and in lung weight adjusted for terminal bodyweight. In main study animals there was a dose- related, increase in BrdU labelling index in terminal bronchioles and centro-acinar alveoli at all exposure levels which generally achieved statistical significance. Recovery phase animals remained similar to controls at all dose levels. There were no macroscopic abnormalities. All main study animals exposed to 10.3mg/m^3 MDI showed an increase in bronchiolitis, thickening of the central acinar region and an increase in the number of alveolar macrophages containing yellow pigment. In animals exposed to 0.93 or 3.88mg/m^{3}MDI 1/5 animals in each group showed bronchiolitis. Recovery phase animals exposed to 10.3mg/m^3 MDI retained alveolar macrophages containing a yellow pigment in the alveolar sacs and in the interstitium and 1/5 of these animals showed evidence of bronchiolitis and central acinar thickening at a reduced severity to that seen in the main study.

1.3 Conclusion

Animals exposed nose-only, for 6 hours per day, 5 days per week over a period of 4 weeks to polymeric MDI at a concentration of 10.3mg/m^3 showed significant changes in most parameters. While many parameters showed little or minimal changes at 0.93, 3.88. There was an exposure-concentration related effect on cell proliferation at all concentrations.

Recovery phase animals showed that most parameters had returned to normal but residual changes consequent to previous increased levels of lung surfactant were still apparent.

A 08

III Project 151

11362

Volume 1

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/6191

POLYMERIC MDI: 28 DAY REPEAT EXPOSURE STUDY IN FEMALE RATS (6 HOURS PER DAY 5 DAYS PER WEEK) AND POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/6191

POLYMERIC MDI: 28 DAY REPEAT EXPOSURE STUDY IN FEMALE RATS (6 HOURS PER DAY 5 DAYS PER WEEK) AND POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

STUDY DETAILS

Sponsor:	International Isocyanate Institute Scientific Office, Floor 9, Bridgewater House Manchester M1 6LT UK
Sponsor Reference:	CO5737
CTL Test Substance Reference Number:	Y00122/021
CTL Study Number:	MR0197

AUTHOR

N J Rattray

DATE OF ISSUE

16 December 1999

STATEMENT OF DATA CONFIDENTIALITY CLAIM

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO THE SPONSOR.

It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of this product or any other product without the written permission of International Isocyanates Institute.

RECISSION OF CONFIDENTIALITY

The information given in this text is not confidential.

DSCuilbert

Scientific Director, International Isocyanate Institute Inc

STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1997). These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

Nicola J Rattray	Nicola J Rattray	16 December 1999
Study Director		Date

QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
22 Jul 98	Protocol	22 Jul 98
24 Sep 98	Exposure, atmosphere generation, atmosphere collection	24 Sep 98
28-29 Sep 98	BrdU preparation, minipump preparation	29 Sep 98
29 Sep 98	Minipump implantation	29 Sep 98
05 Oct 98	Lung lavage	05 Oct 98
06 Oct 98	Post mortem	06 Oct 98
06 Oct 98	Derivitisation of MDA	08 Oct 98
03 Nov 98	Cell counting, slide preparation	03 Nov 98

In addition, procedure inspections associated with this type of study were made as follows:

06 Aug 98	Clinical observations, bodyweights	06 Aug 98
14 Aug 98	Atmosphere analysis	17 Aug 98

The haematology and clinical chemistry individual animal data contributions have been auditied by ZENECA Pharmaceuticals Quality Assurance as follows:

23 Jul 98	23 Jul 98
21 Dec 98	21 Dec 98
06 Jan 99	11 Jan 99

Facilities and process based procedures associated with this study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, MR0197.

G P Fuller ..... 16 December 1999

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
N J Rattray	Study Director
P M Hext	Study Reviewer
S Duffell	Clinical Pathologist
A Soames	Histologist
C Bowling	Electron Microscopist
J Foster	Pathologist
M Crawley	Chemical Analyst
I Pate	Statistician
S Millward	Study Technician
K P Jackson	Principal Investigator
	Zeneca Pharmaceuticals
	Alderley Park Macclesfield Cheshire
	SK10 4TG UK

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FIGURES

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APPENDICES

2. INTRODUCTION

The purpose of this study was to investigate the effect on the rat lung of exposure to polymeric MDI aerosol for 6 hours per day, 5 days per week, over a period of 4 weeks. Since deposited aerosol is retained within the lung for a time period following exposure, additional groups of animals were retained for a period 30 days following exposure for investigation of recovery or progression of lung responses. A detailed investigation of the response included assessment of cell proliferation, assessment of any change in the lung lining fluid and associated free cell population, and light and electron microscopy.

The purpose of this study was to assess the toxicity to female rats of polymeric MDI after inhalation exposure for 6 hours per day, 5 days per week, over a 4 week period and to monitor recovery from any effects observed over a further 30 days. The information obtained will help to determine whether the low carcinogenic potential of MDI found in long-term bioassay (Reuzel et al 1994) may have its origin in non-genotoxic processes occurring in the respiratory tract as an early response in chronic exposures.

2.1 Regulatory guidelines

This study was not conducted to any specific regulatory guidelines but conformed in part with the test guidelines of OECD (OECD 1981), of EEC (1992) and EPA (1988).

2.2 Justification for test system selection

The Alpk:APfSD strain of rat was used because of the substantial background data available for this strain, in this Laboratory, relating to studies of this type.

2.3 Selection of atmospheric concentrations

The nominal atmospheric concentrations of 1, 4 and or 10mg/m^3 were selected in consultation with the Sponsor on the basis of findings from previous sub-chronic and chronic inhalation studies with this test material.

2.4 Study dates

The study was initiated on 15 July 1998. The first data collected on this study (trial generation) was 14 August 1998. The experimental phase started on 9 September 1998 and was completed 24 February 1999.

2.5 Data storage

An original report, all raw data, samples and specimens pertaining to this study, with the exception of clinical pathology raw data, are retained in the Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Clinical pathology raw data are stored in the Safety of Medicines Archives, Zeneca Pharmaceuticals, Alderley Park.

3. TEST SUBSTANCE

3.1 Test substance

Name:	Polymeric MDI (Suprasec 5005)
Source:	Ex Hill House International.
Colour:	Dark brown
Physical state:	Liquid
Batch reference number:	Lab Ref 982
CTL test substance reference number:	Y00122/021
Polymeric composition:	
%Di/MDI	44.11
%Tri/MDI	22.94
Storage conditions:	Ambient temperature in the dark under nitrogen.

The sample was tested as supplied and was used within the expiry date. A certificate of analysis is retained in the CTL archive.

4. EXPERIMENTAL PROCEDURES

4.1 Atmosphere concentration

4.1.1 Trial generation

Trial generations were carried out prior to the start of exposure in order to:

i) determine the appropriate generation system and conditions

ii) determine that required concentrations could be achieved

iii) obtain data on the aerodynamic particle size of the atmosphere generated

4.1.2 Generation conditions

A diagram of the atmosphere generation system is shown in Figure 1. Each test atmosphere was generated using a glass concentric-jet atomiser and a cyclone. The test substance was pumped to the atomiser using a peristaltic pump. Clean, dry air (dried and filtered using equipment supplied by Atlas-Copco, Sweden) was passed through the atomiser at nominal flow rates of 9/14.5, 2/4 or 14/17l/minute (at normal temperature and pressure) for the 1, 4 or 10mg/m^3 concentrations respectively and carried the atmosphere to each of the exposure chambers (internal volume of 46 litres). Diluting air was added directly to the exposure chambers at flow rates of 35/40, 40/41,or 30/40 l/minute respectively for the 1, 4 or 10mg/m^3 concentrations. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flowmeters and were altered as necessary to maintain the target concentrations.

4.2 Atmosphere sampling and analysis

4.2.1 Gravimetric concentrations

The particulate concentration of each test atmosphere, close to the animals' breathing zone, was measured gravimetrically at least twice per day. This was done by drawing each test atmosphere, at a known flow rate, for a known time, through a 25mm diameter, glass fibre filter, Gelman Sciences Limited, Northampton, UK). The atmospheric concentration of polymeric MDI was determined gravimetrically for each exposure level by weighing the

material collected on the filters and this figure was taken as the concentration of polymeric MDI in the test atmospheres.

The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{Concentration (mg/l)} = \frac{\text{post wt - pre wt (mg)}}{\text{time (minute) x airflow (l/minute)}}$$

pre wt = weight of filter prior to sampling
post wt = weight of filter after sampling

4.2.2 Analysed concentration

The analysed concentration of each test atmosphere, close to the animals' breathing zone, was measured daily by drawing each test atmosphere, at a known flow rate, for a known time, through a 25mm diameter, Institute of Occupational Medicine (IOM) sampler SKC Ltd., Blandford Forum, Dorset, UK., housed in an IOM open-faced filter holder. Prior to sampling, the IOM filters were impregnated with 1-(2-methoxyphenyl) piperazine (1,2-MP) reagent. Following sampling the filters were removed, weighed, and the collected aerosol extracted/desorbed prior to analysis by HPLC.

4.2.3 Atmospheric characterisation

The characterisation of the test atmospheres was carried out by analysing the material collected on the pre-impregnated IOM filters. The analytical method is given in Appendix A. Full details of reagents, filter impregnation, extraction/desorption and analysis methods are provided in Hext (1996).

4.2.4 Aerodynamic particle size distribution

The aerodynamic particle size distribution of each test atmosphere was measured once during each day for the first week, then once during each subsequent 5 day exposure period using a Marple Cascade Impactor (supplied by Schaefer Instruments Limited, Wantage, Oxon, UK) which aerodynamically separates airborne particles into pre-determined size ranges. The amount of aerosol, by weight, in each size range, was then used to calculate the aerodynamic particle size distribution of the aerosol. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data.

Using this regression line, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated. Definitions of particle size are given in Appendix B.

4.3 Experimental design

4.3.1 Animals

Species:	Rat
Strain:	Alpk: AP$_f$SD
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK.
Sex/number:	146 females
Specification (age)	Approximately 7-9 weeks old at start of study.

4.3.2 Accommodation and husbandry

On arrival in the inhalation unit, the rats were housed 5 per cage in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.

The rats were transferred to clean cages and racks as necessary during the study.

The animal room was designed to give the environmental conditions shown as follows:

Temperature:	22±3°C
Relative humidity:	30-70%
Air:	At least 15 changes/hour
Light cycle:	Artificial giving 12 hours light, 12 hours dark

Both temperature and relative humidity were measured and recorded daily. In general, the recorded values were within the specified ranges and any deviations that were observed are considered not to have affected the integrity of the study.

Diet, RM1, supplied by Special Diet Services Limited, Witham, Essex, UK and mains water, supplied by an automatic system were available *ad libitum*, except during exposure.

Each batch of diet is routinely analysed for composition and for the presence of contaminants. Water is also periodically analysed for the presence of contaminants. No contaminants were

found to be present in the diet or water at levels considered to be capable of interfering with the purpose or outcome of the study. Certificates of analyses are retained in the CTL Archives.

4.3.3 Acclimatisation

The animals were housed under the experimental conditions for approximately 2 weeks at CTL, prior to the start of the study.

4.3.4 Animal randomisation and identification

The animals were allocated to the groups using the method shown in Appendix C. The individual animal numbers for each cage were determined by group and replicate and are shown in the rack plan (Appendix D). In addition, after acclimatisation, the rats were familiarised to the restraint tubes prior to exposure for 2, 4 and 6 hours on days -3, -2 and -1 respectively. On the front of each cage of animals was a card identifying the contained animals by exposure concentration, group number, individual number, sex and study. The cage cards were colour-coded to correspond with exposure group.

4.3.5 Atmospheric concentrations and exposure groups

The study consisted of one control group and three treatment groups, with 15 or 20 females per group for the main study and 15 or 20 females per group for the recovery phase.

The experimental numbers and the groups to which these rats were assigned are shown overleaf.

ANIMAL NUMBERS AND TREATMENT GROUPS

Examination on day 1 *post* exposure

Test group	Colour code	Conc. (mg/m^3)	Animal Nos for lung lavage sample 1 for enzymes and cell analysis	Animal Nos for lung lavage sample 2 for phospholipid analysis	Animal Nos for lung lavage sample 3 for chemical analysis of macrophages)	Animal Nos for pathology
1.1	blue	0	1-5	6-10	11-15	16-20
1.2	green	1	21-25	26-30		31-35
1.3	yellow	4	36-40	41-45		46-50
1.4	red	10	51-55	56-60	61-65	66-70

Nos = numbers

Examination on day 30 *post*-exposure.

Test group	Colour code	Conc. (mg/m^3)	Animal Nos for lung lavage sample 1 for enzymes and cell analysis	Animal Nos for lung lavage sample 2 for phospholipid analysis	Animal Nos for lung lavage sample 3 for chemical analysis of macrophages)	Animal Nos for pathology
2.1	blue	0	101-105	106-110	111-115	116-120
2.2	green	1	121-125	126-130		131-135
2.3	yellow	4	136-140	141-145		146-150
2.4	red	10	151-155	156-160	161-165	166-170

Nos - numbers

4.3.6 Exposure system

The rats were exposed nose-only to the test atmospheres in restraining tubes supplied by Battelle, Geneva, Switzerland. These were inserted into a PERSPEX exposure chamber and the relative position of each rat in the exposure chamber was changed daily to ensure uniform exposure (Appendix E). The chamber was covered with an aluminium cone and stood on an aluminium base. The atmosphere was shown to be acceptably stable over approximately 30 minutes before exposure of the test animals. During this period the holes of the exposure chamber were plugged. The animals were exposed for 6 hours per day, 5 days per week, over a period of 4 weeks.

The temperature and relative humidity within each chamber was measured at frequent intervals during the exposure using a portable, digital temperature and relative humidity monitor. Within the chambers, the temperature ranged between 17.6 and 28.2°C and the relative humidity ranged between 4 and 58%. The daily averages are displayed in Appendix F

Control animals were exposed to air only, but were otherwise treated in a similar manner to the test animals.

4.3.7 Duration of exposure

The animals were first exposed on 9 September 1998 and were exposed for 6 hours per day, 5 days a week over period of 4 weeks.

4.3.8 Duration of recovery period

The animals designated for the recovery phase were exposed as for the main study then monitored for a further 30 days after the last exposure.

4.4 Clinical observations

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure, they were observed frequently and, at the end of the 6-hour exposure period, each rat was examined. Detailed clinical observations, included the finding of no abnormalities detected were recorded on day 1 following exposure, weekly for the remainder of the study and prior to scheduled termination.

4.5 Bodyweights

The bodyweight of each rat was recorded on day 1 before the first exposure and thereafter once a week and prior to termination.

4.6 BrdU labelling indices

Animals designated for 'S' phase analysis were implanted subcutaneously, on the dorsal surface, with osmotic Minipumps (Alzet model 2ML1), containing 15mg/ml BrdU, 7 days prior to scheduled termination.

4.7 Investigations *post mortem*

4.7.1 Termination

All rats were killed by exsanguination (cardiac puncture) under terminal anaesthesia induced by euthetal.

4.8 Examination of the lung lavage

4.8.1 Cytology and clinical chemistry analysis

The lungs were lavaged 6 times *in situ*. The first lavage sample from each designated animal was centrifuged separately and the supernatant retained for the measurement of clinical chemistry parameters; the supernatants from the 5 subsequent lavages were discarded. The pooled cell pellet from all 6 lavages was retained and resuspended in 1ml of phosphate buffered saline on ice for the cytological examinations.

The total cell count was carried out on 100µl of the final cell suspension diluted 1/10 by the addition of 900µl of phosphate buffered saline prior to counting. The differential cell count was carried out on a 50µl quantity of the total cell suspension (well mixed by gentle hand shaking) from each animal and the slide prepared on a *Cytospin 2* machine. The prepared slides were fixed in methanol for 10 minutes and stained using the Romanowsky method. A manual differential count was performed dividing the cell population into three cell types: alveolar macrophages, polymorphonuclear leucocytes and lymphocytes/other cell types. This latter group comprised mononuclear cells lacking plentiful cell cytoplasm as seen in alveolar macrophages.

The supernatant from the first lavage was retained on ice for examination of clinical chemistry parameters namely protein, lactate dehydrogenase activity, alkaline phosphatase activity and N-acetyl glucosaminidase activity.

4.8.2 Phospholipid analysis

The supernatant from the first 2 lavages was pooled, centrifuged, and separated from the cell pellet. The supernatant sample was stored at -20°C prior to analysis for phosphatidylcholins. The remaining 4 lavages were pooled, centrifuged and the supernatant discarded. The cells from these 4 lavages together with the cell pellet from the first 2 lavages were resuspended in

phosphate buffered saline, re-centrifuged and all supernatant discarded. The pelleted cells were stored at -20°C prior to analysis for intracellular phosphatidylcholins.

The method of analysis was based on that of Takayama M et al. 1977.

The reagent kit was supplied by Roche Diagnostics, Bell Lane, Lewes, East Sussex, BN7 1LG (catalogue number 691844) and was used as per instructions. The absorbance of the standards and samples was read at 500nm and compared with reagent blank. The reaction was linear up to a concentration of 1000mg phospholipid/dl and any samples which exceeded this were appropriately diluted. The samples were analysed in a random order.

Phospholipid concentration was calculated as follows:

The concentration of the choline chloride standard solution was 54.1mg/dl corresponding to 300mg phospholipids/dl [Mr774] (=3.88mmol/l).

$$\text{Concentration} = 3.88 \times \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \quad [\text{mmol/l}]$$

4.9 Lung weights

Lungs (with trachea attached but larynx removed) were weighed from animals designated for pathology.

4.10 Macroscopic examination

All animals designated for pathology were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera, brain and cranial cavity.

4.10.1 Tissue submission

The following tissues were taken from all animals designated for pathology and preserved in 10% neutral buffered formol saline.

head*
larynx*
trachea*
lungs
mediastinal lymph nodes*
jejunum

Tissues marked * were stored.

4.10.2 Tissue processing

10% neutral buffered formol saline was infused into the lungs via the trachea using a syringe. Samples of the lung tissue for electron microscopy were removed after fixation and transferred to a glutaraldehyde solution for *post*-fixation.

The lung and jejunum from the BrdU implanted animals were processed for subsequent immunostaining and additional sections of lung were sectioned at 5µm, and stained with haematoxylin and eosin.

4.10.3 Microscopic examination

Sections stained with haematoxylin and eosin were examined by light microscopy.

The BrdU labelling index in the terminal bronchioles was evaluated separately from that in the centro-acinar bronchiolar regions. For each area, a sufficient number of regions were assessed to give an approximate total number of cells for each area for each animal of 2000 cells. The number of bronchiolar and alveolar cells that entered DNA synthesis over the labelling period were evaluated as a ratio against the total number of cells.

4.10.4 Light microscopy of lavage pellet

Macrophages in the preparations from the lavage fluid used for differential counts were examined for the presence of vacuoles. One preparation from each animal was examined. The number of macrophages with distinct vacuoles, termed foamy macrophages, was estimated subjectively over the whole slide.

4.10.5 Electron microscopy

The glutaraldehyde *post*-fixed samples of lung tissue were embedded in epoxy resin and semithin sections were prepared. Toluidine blue stained semithin slides were examined and assessed. From selected areas ultrathin sections were prepared and examined by electron microscopy.

5. DATA EVALUATION

All data, with the exception of foamy macrophages, were evaluated using analysis of variance and/or covariance using the GLM Procedure in SAS (1989) package. Details of the statistical methods used are given in Appendix G.

6. RESULTS

6.1 Atmosphere analysis

6.1.1 Particulate concentrations

The study mean (mean of daily means) concentrations [± standard deviations (SD)] determined gravimetrically were as follows:

Group	Target Polymeric MDI concentration mg/m^3	Total particulate concentration (mg/l) Mean ± SD
2	1	0.93 ± 0.39
3	4	3.88 ± 0.87
4	10	10.3 ± 2.31

6.1.2 Analysed concentrations

The overall mean analysed concentrations are shown overleaf and the daily mean concentrations are shown in Table 1. While some variability between the Di, Tri and gravimetric analyses is evident, overall there is good correlation between analysed and gravimetric concentrations. The sum of the Di+Tri polymers will always be less than the gravimetric total because polymeric MDI is 40-50% Di, 20-25% Tri and the rest is higher homologues.

Total particulate ($mg.m/^3$)	Di-MDI ($mg.m/^3$)	Tri-MDI ($mg.m/^3$)	Di/Tri Ratio
0.93	0.41	0.2	2.07
3.88	1.5	0.73	2.1
10.3	3.84	1.77	2.25

The analysed Di:Tri ratio is close to that in the sample supplied. The gravimetric concentrations (Section 6.1.1) are considered to represent the true overall concentrations of polymeric MDI and hence will be used to subsequently describe the test groups (Table 1).

6.1.3 Aerodynamic particle size distribution table

The mean aerodynamic particle size distribution of the total particulate was extrapolated from the data and the overall study means are as follows:

Group	Total particulate concentration mg/m^3	Median size (MMAD) (µm)	GSD
2	0.93	1.14	2.47
3	3.88	1.61	1.95
4	10.3	1.09	1.68

Individual values are given in Table 2.

6.2 Clinical observations

6.2.1 Mortality

There were no deaths.

6.2.2 Observations during exposure

Throughout the exposure period animals in all groups were wet and stained around the nose with the incidence of these findings increasing with the time of the exposure. These abnormalities are generally associated with restraint.

6.2.3 Observations during exposure and recovery phase

Clinical changes during the main study were confined to 1 animal exposed to 10.3mg/m^3. These observations were cold, hunched appearance, pale, and piloerection and occurred on days 26/27 of the study. In view of the late onset of these changes and that no other animal in the group showed any clinical abnormalities it is considered that they were unrelated to exposure to MDI. There were no clinical changes during the recovery phase of the study (Table 3).

6.3 Bodyweights

Bodyweights in all groups were comparable to controls throughout the exposure and recovery periods (Figures 2 - 3, Table 4).

6.4 Investigations *post mortem*

6.5 Lung lavage

6.5.1 Examination of lung lavage - cytology

The total cell count, alveolar macrophages, polymorphonuclear leucocytes and lymphocyte/other cells were counted in the lung lavage fluid. There was a statistically significant increase in both the total number of cells counted and of alveolar macrophages in the main study at 10.3mg/m^3, and a slight (but not statistically significant) increase in these counts at 3.88mg/m^3. Polymorphonuclear leucocytes and lymphocyte/other cells showed a statistically significant, dose-related increases at 3.88 and 10.3mg/m^3. At the end of the recovery phase, cell counts in exposed animals had returned to normal numbers and distribution (Figure 4, Table 5).

6.5.2 Examination of lung lavage - clinical chemistry

Total protein, alkaline phosphatase, lactate dehydrogenase and N-acetyl glucosaminidase activities were measured in the lung lavage fluid. Total protein and alkaline phosphatase activity were statistically significantly increased in main study animals exposed to 10.3mg/m^3 MDI. Lactate dehydrogenase and N-acetyl glucosaminidase activities at this exposure level were comparable to control values. All other treated groups in the main study and at the end of the recovery phase showed values similar to controls (Figure 5, Table 5).

6.5.3 Examination of lung lavage - phosphatidylcholins

The level of phosphatidylcholins was measured in the lung lavage supernatant and in the cell pellet. In the main study there was an increase in phospholipids in both the supernatant lung lavage fluid and in the lung lavage cell pellet at 10.3mg/m^3. The cell pellet phospholipid concentration was also increased at 3.88mg/m^3. No differences from control values were seen at the end of the recovery phase (Figure 6, Table 5).

6.6 Lung weights

Main study animals exposed to 10.3mg/m^3 showed an increase in lung weight and in lung weight adjusted for terminal bodyweight. The lung weights of all other treated groups were comparable to control values (Figure 7, Table 6).

6.7 BrdU labelling indices

In main study animals there was a statistically significant, dose-related, increase in BrdU labelling index in terminal bronchioles at all exposure levels. A similar increase was seen in centro-acinar alveoli in animals exposed to 10.3 and 3.88mg/m^3 MDI. Animals exposed to 0.93mg/m^3 MDI showed an increase in labelling index but this did not achieve statistical significance. At the end of the recovery phase animals labelling indices were similar to controls at all exposure concentrations (Figure 8 Table 7).

6.8 Pathology examination

6.8.1 Macroscopic examination

There were no macroscopic abnormalities.

6.8.2 Microscopic examination

All main study animals exposed to 10.3mg/m^3 MDI showed an increase in bronchiolitis, thickening of the central acinar region and an increase in the number of alveolar macrophages containing yellow pigment. In animals exposed to 3.88mg/m^3, 1/5 animals showed thickening of the central acinar region and bronchiolitis and 1/5 animals exposed to 0.93mg/m^3 showed bronchiolitis.

After the recovery phase, alveolar macrophages containing a yellow pigment were present in the interstitium in all animals that had been exposed to 10.3 mg/m^3 MDI but were absent in animals exposed to 0.93 or 3.88mg/m^3. In addition, 1/5 animals exposed to 10.3mg/m^3 MDI still had bronchiolitis and centro-acinar thickening but at a reduced severity and distribution to that seen in the main study (Table 8).

6.8.3 Light microscopy of lung lavage cell pellet

Main study animals exposed to 10.3mg/m^3 MDI showed a large increase in the number of macrophages containing vacuoles (foamy macrophages) when compared to controls with the

effect being of similar incidence but reduced severity in animals exposed to 3.88mg/m^3 MDI. All other treated groups were comparable to controls (Table 9).

6.8.4 Electron microscopy

Animals on the main study exposed to 10.3mg/m^3 MDI showed a slight thickening of the interstitial alveolar wall in 3/5 animals. The thickening in the centro-acinar region was due to thickening of the interstitium, which is partly attributable to the absorption of alveolar macrophages and partly due to excess collagen. In alveolar Type II cells, minimal dose-related increases were noted in the size of the lamellar bodies (increases in the number of lamellar bodies were considered not to be compound-related).

Compound-related increases in the levels of surfactant were noted in the alveolar macrophages and lumen. In the alveolar macrophages, minimal to slight increases in lamellar surfactant were associated with minimal and moderate increases in amorphous surfactant in animals exposed to 10.3mg/m^3 MDI. In the alveolar lumen, minimal to moderate increases in cell debris were noted in animals exposed to 10.3 or 3.88mg/m^3 MDI. Associated with these increases in cell debris were increases in the amount of crystalline and lamellar surfactant. Not all of the above changes showed a dose-response relationship.

In the recovery phase 1/5 of the animals exposed to 10.3mg/m3 MDI showed residual thickening of the interstitial alveolar wall. Macrophages showed minimally increased levels of amorphous surfactant over controls but this change was reduced in comparison to that observed on the main study. In addition surfactant and debris levels were also reduced in the alveolar lumen when compared to the main study but were still higher than in the controls (Table 10).

7. DISCUSSION

The purpose of this study was to assess the toxicity to female rats of polymeric MDI after inhalation exposure for 6 hours per day, 5 days per week, over a period of 4 weeks and to monitor recovery from any effects observed over a further 30 days; this was successfully accomplished. The health of the rats was satisfactory and there was no evidence of disease or infection which might have compromised the interpretation of the findings.

The characteristics of the test atmospheres were acceptable with regard to the aims of the study.

Clinical findings during exposure of wet fur and stained around the nose are generally associated with restraint and were considered to be of no toxicological significance. There were no other clinical changes considered to be related to exposure to MDI and no effects on bodyweights.

Analysis of bronchoalveolar lavage fluid showed changes in the majority of parameters at 10.3 mg/m^3. Total cell count was increased statistically significantly. This was accounted for by increases in alveolar macrophages, polymorphonuclear leucocytes (PMN's) and lymphocytes/other cell types. Additionally, the percentage of each of the cell types in relation to the total had changed compared with controls. Whereas macrophages constitute over 99% of cells in the control rat lung, this was reduced to 84% in rats exposed to 10.3 mg/m^3 MDI , with PMN's and lymphocytes/other cell types representing the remainder. Small changes were also seen in these cell parameters at 3.88 mg/m^3. At both concentrations increased numbers of "foamy" macrophages were seen and correlate with the increased analysed phospholipid content of the lavaged cells. Following the recovery period all parameters had returned to the normal range of values.

Some lavage fluid parameters showed moderate increases at 10.3 mg/m^3 only. Following the recovery period these parameters had returned virtually to normal although a few individual animals appeared to have retained increased protein and LDH values.

In the terminal bronchioles there was a concentration-related increase in cell proliferation at all concentrations. A similar pattern was seen in the centro-acinar alveoli but the apparent small increase at 0.93 mg/m^3 did not achieve statistical significance. Combination of both regions for comparison with the results from the 2-week study showed statistically significant increases at all concentrations. A return to control proliferative rates was evident at the end of the recovery period.

Compound-related increases in the levels of surfactant were noted in the alveolar macrophages and lumen. In the alveolar macrophages, minimal to slight increases in lamellar surfactant

were associated with minimal and moderate increases in amorphous surfactant in animals exposed to 10.3mg/m3 MDI. In the alveolar lumen, minimal to moderate increases in cell debris were noted in animals exposed to 10.3 or 3.88mg/m3 MDI. Associated with these increases in cell debris were compound-related increases in the amount of crystalline and lamellar surfactant. Not all of the above changes showed a dose-response relationship. In the recovery phase 1/5 of the animals exposed to 10.3mg/m3 MDI showed residual thickening of the interstitial alveolar wall. Macrophages showed minimally increased levels of amorphous surfactant over controls but this change was reduced in comparison to that observed on the main study. In addition surfactant, surfactant and debris levels were also reduced in the alveolar lumen when compared to the main study but were still higher than in the controls.

The increased amounts of material, considered to be pulmonary surfactant, in the lumen and alveolar macrophages together with moderate increases in alveolar type II cell lamellar bodies (numbers and size) were consistent with the increased phospholipids measured in the cells and lavage fluid and indicate a mild disturbance of surfactant homeostasis

The findings in this study are consistent with exposure to a mildly irritant aerosol.

8. CONCLUSION

Animals exposed nose-only, for 6 hours per day, 5 days per week over a period of 4 weeks to polymeric MDI at a concentration of 10.3mg/m^3 showed significant changes in most parameters. While many parameters showed little or minimal changes at 0.93, 3.88. There was an exposure-concentration related effect on cell proliferation at all concentrations.

Recovery phase animals showed that most parameters had returned to normal but residual changes consequent to previous increased levels of lung surfactant were still apparent.

9. REFERENCES

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GLOSSARY FOR FIGURES 2-8

Key:

The nominal concentrations of 1, 4 or 10mg/m^3 quoted on the Figures equate to the achieved concentrations of 0.93, 3.88 or 10.3mg/m^3 respectively.

Mg**3 - mg/m^3

Figures 4-8

Bars represent mean +95% confidence limit.

Rec. recovery phase

FIGURE 1 - DIAGRAM OF ATMOSPHERE GENERATION SYSTEM

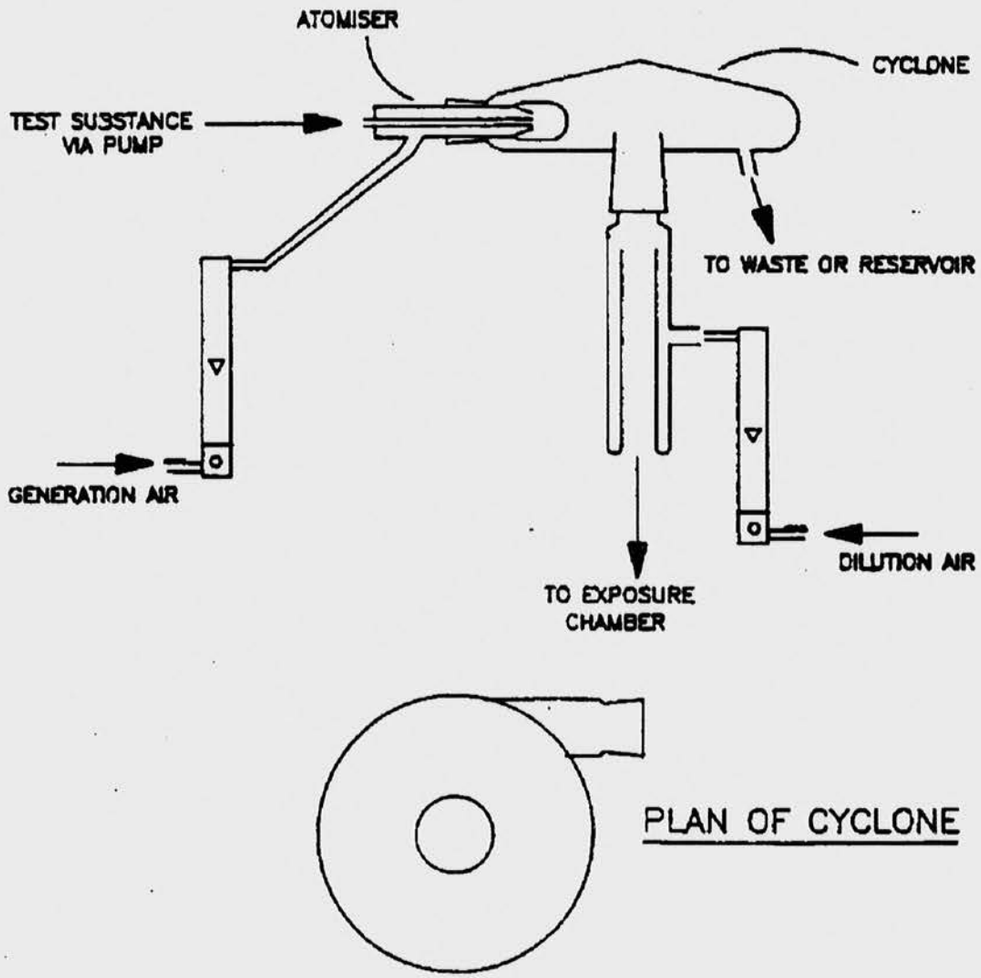


FIGURE 2

FIGURE 2 - GROUP MEAN BODYWEIGHT VERSUS TIME
MAIN STUDY

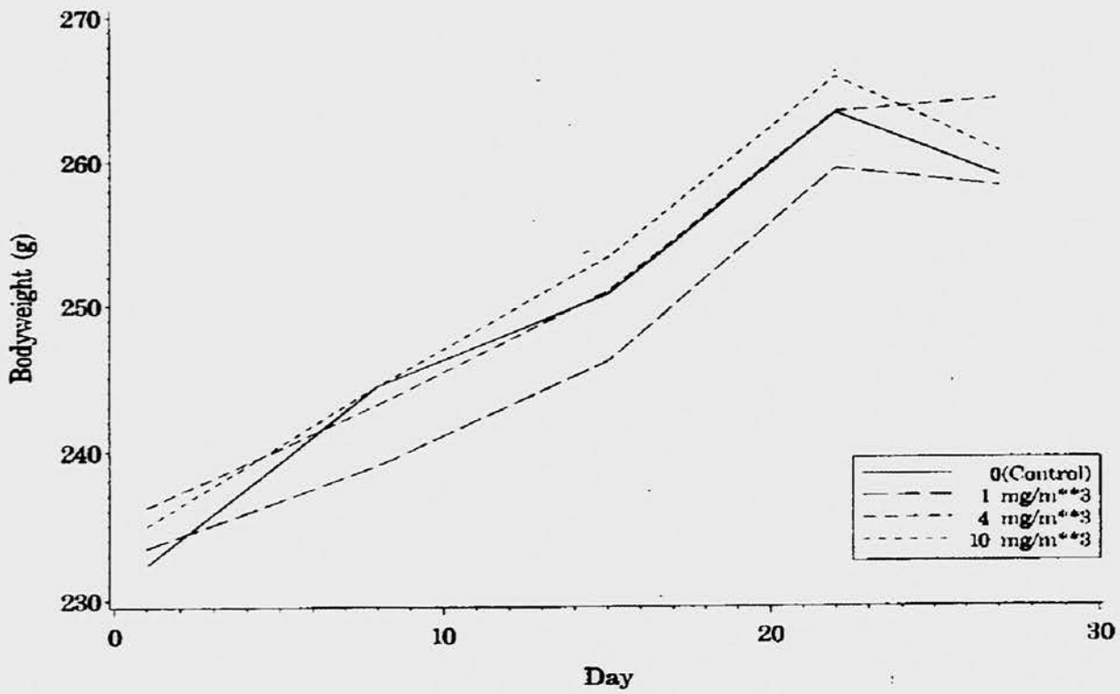


FIGURE 2 - GROUP MEAN BODYWEIGHT VERSUS TIME RECOVERY PHASE

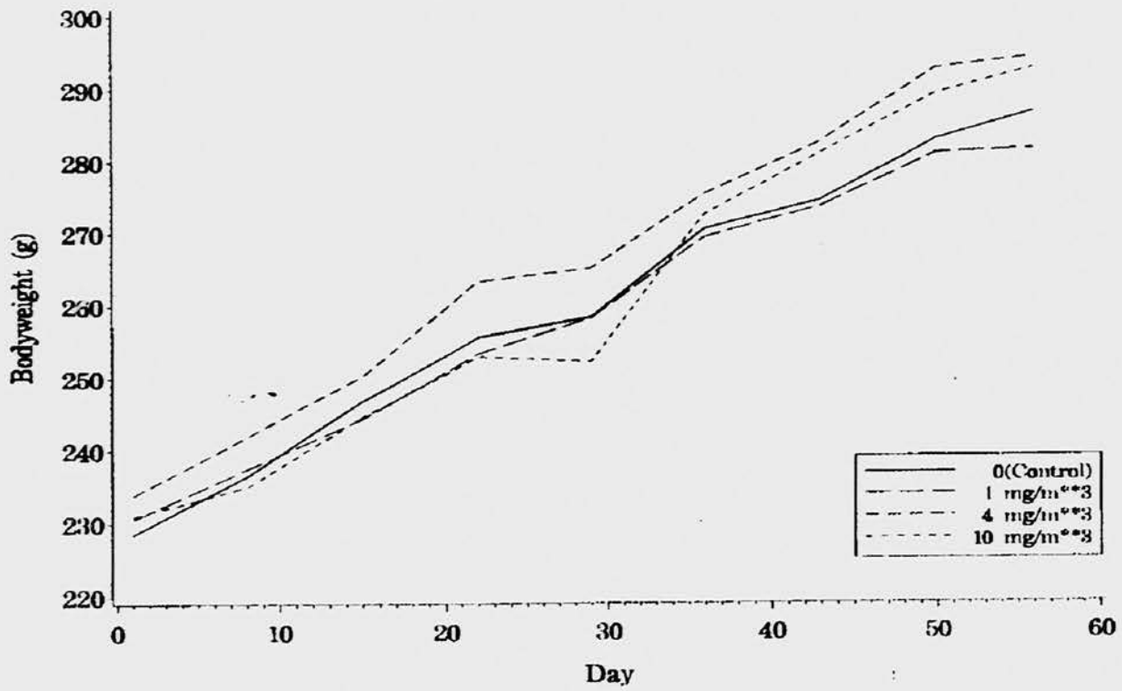


FIGURE 3 - STATISTICAL ANALYSIS OF BODYWEIGHT ADJUSTED FOR INITIAL WEIGHT MAIN STUDY

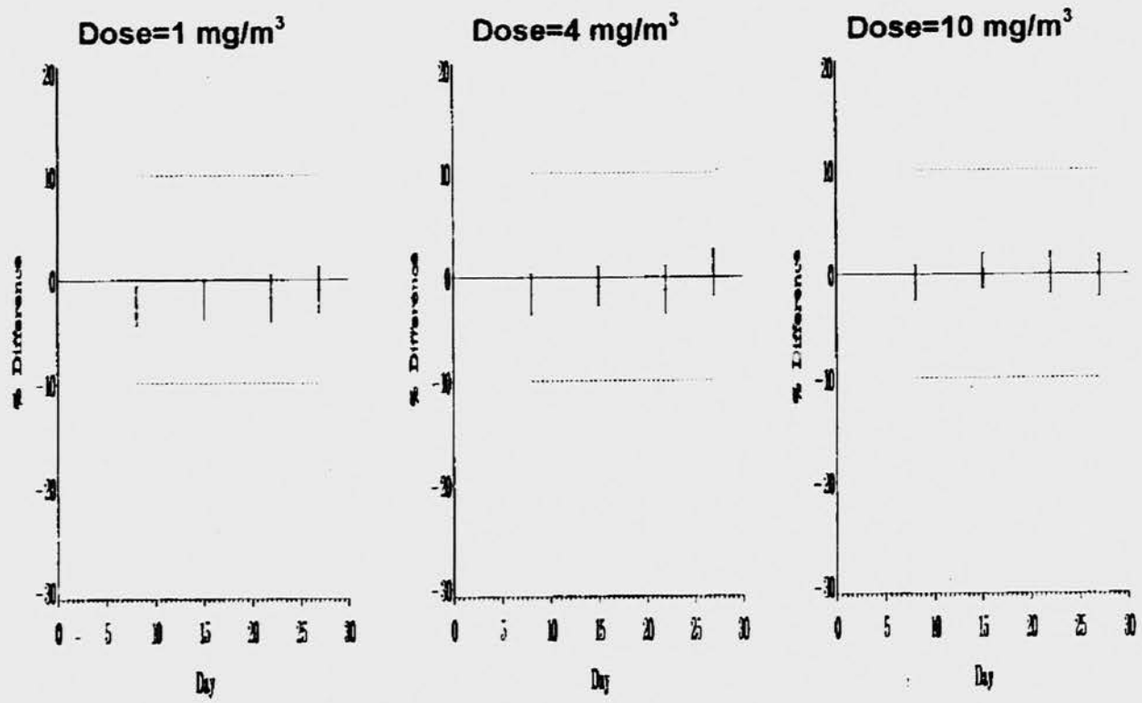


FIGURE 3 - STATISTICAL ANALYSIS OF BODYWEIGHT ADJUSTED FOR INITIAL WEIGHT RECOVERY PHASE

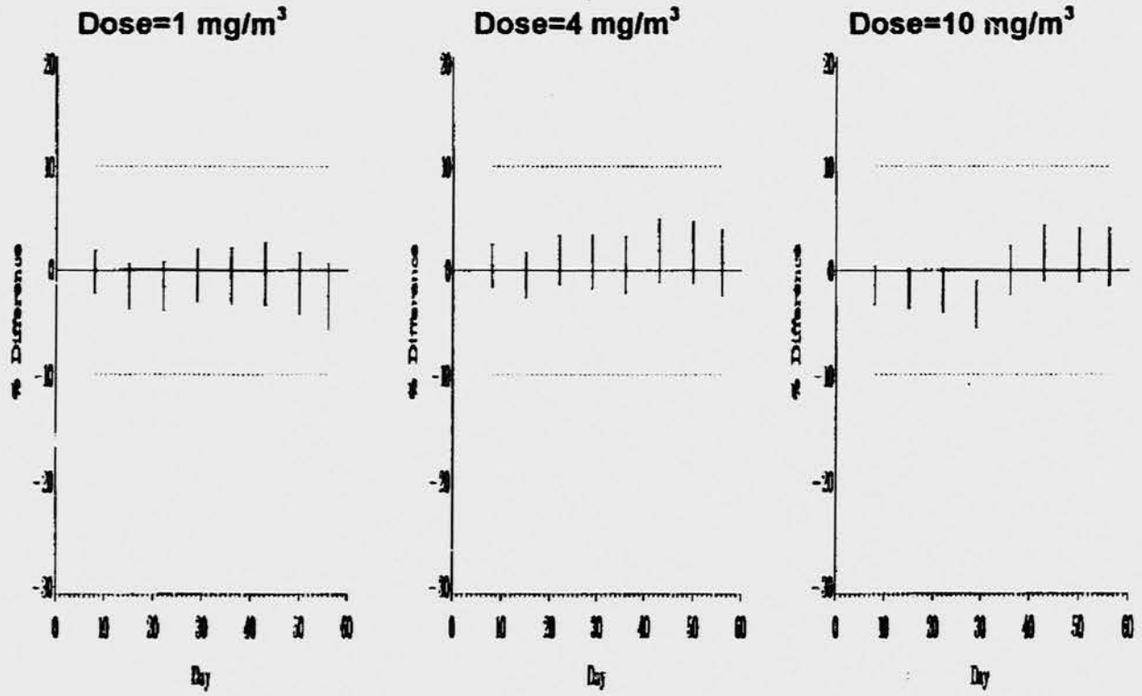


FIGURE 4 - LUNG LAVAGE FLUID
GROUP MEAN TOTAL CELL COUNT ($x10^6$/ml)

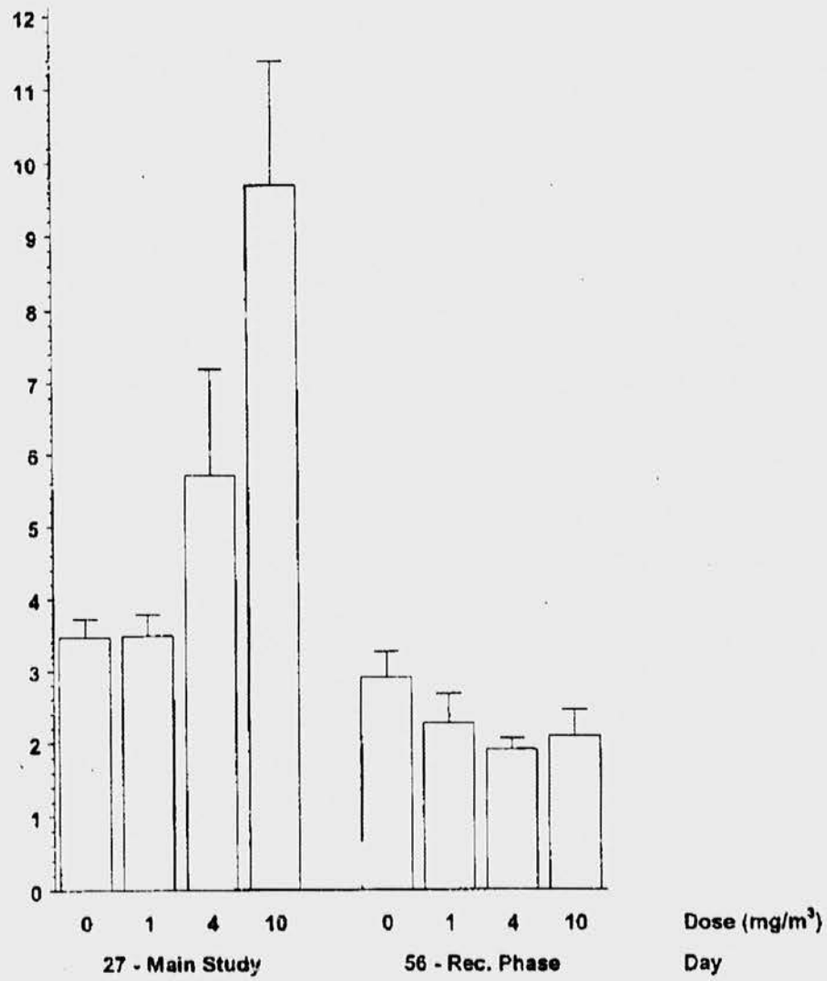


FIGURE 4 - LUNG LAVAGE FLUID
GROUP MEAN ALVEOLAR MACROPHAGE COUNT ($x10^6$/ml)

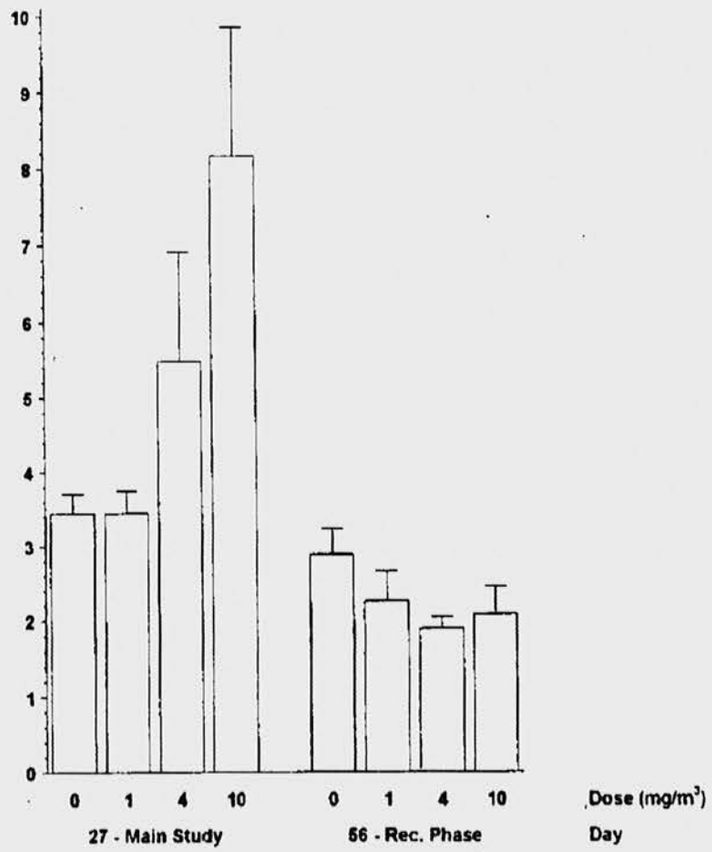


FIGURE 4 - LUNG LAVAGE
GROUP MEAN POLYMORPHONUCLEAR LEUCOCYTE CELL COUNT ($x10^6$/ml)

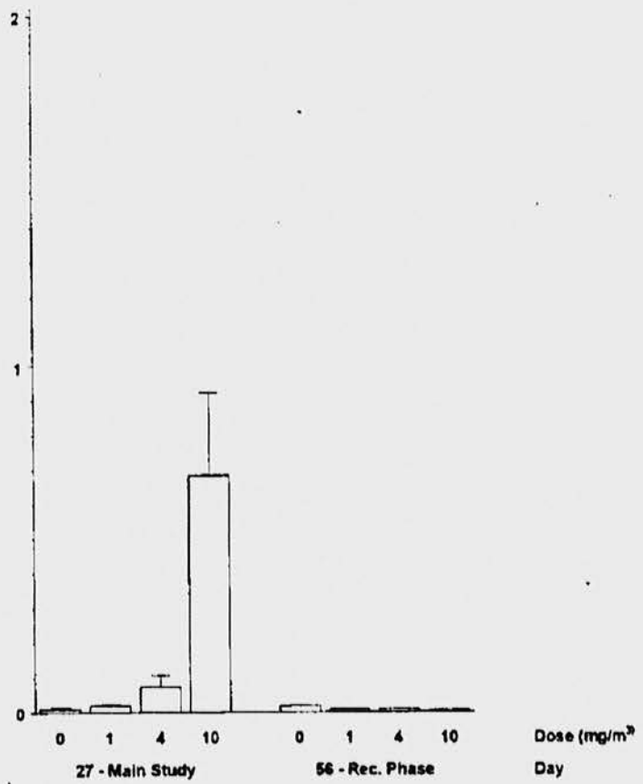


FIGURE 4 - LUNG LAVAGE FLUID
GROUP MEAN LYMPHOCYTE/OTHER COUNT ($x10^6$/ml)

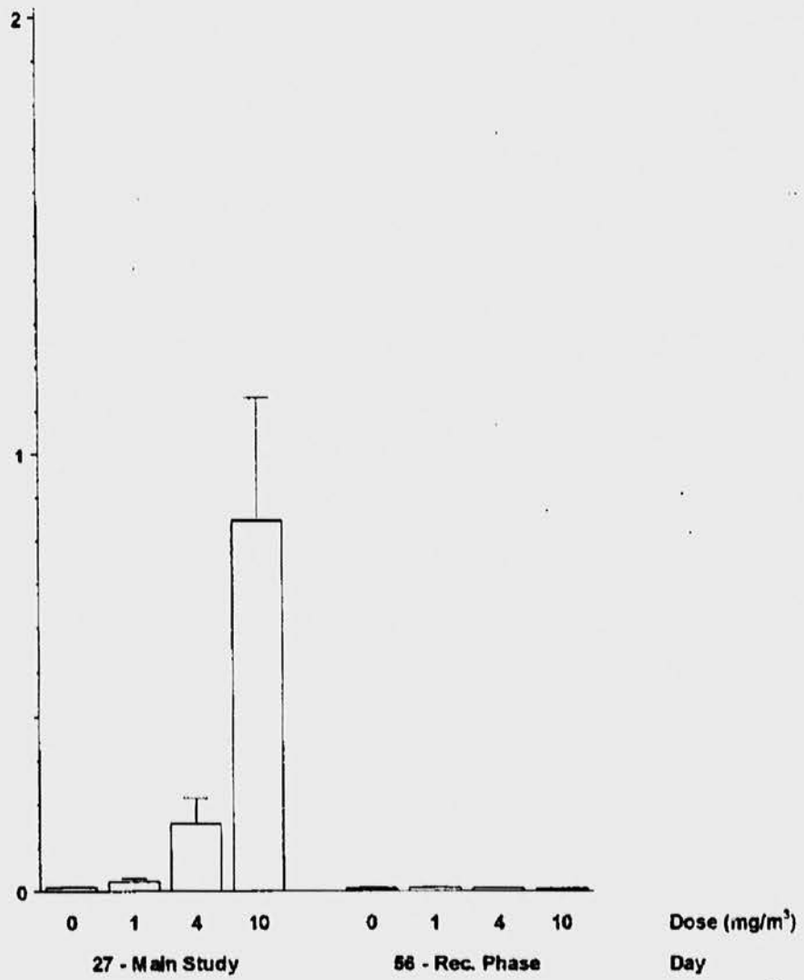


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN TOTAL PROTEIN (mg/dl)

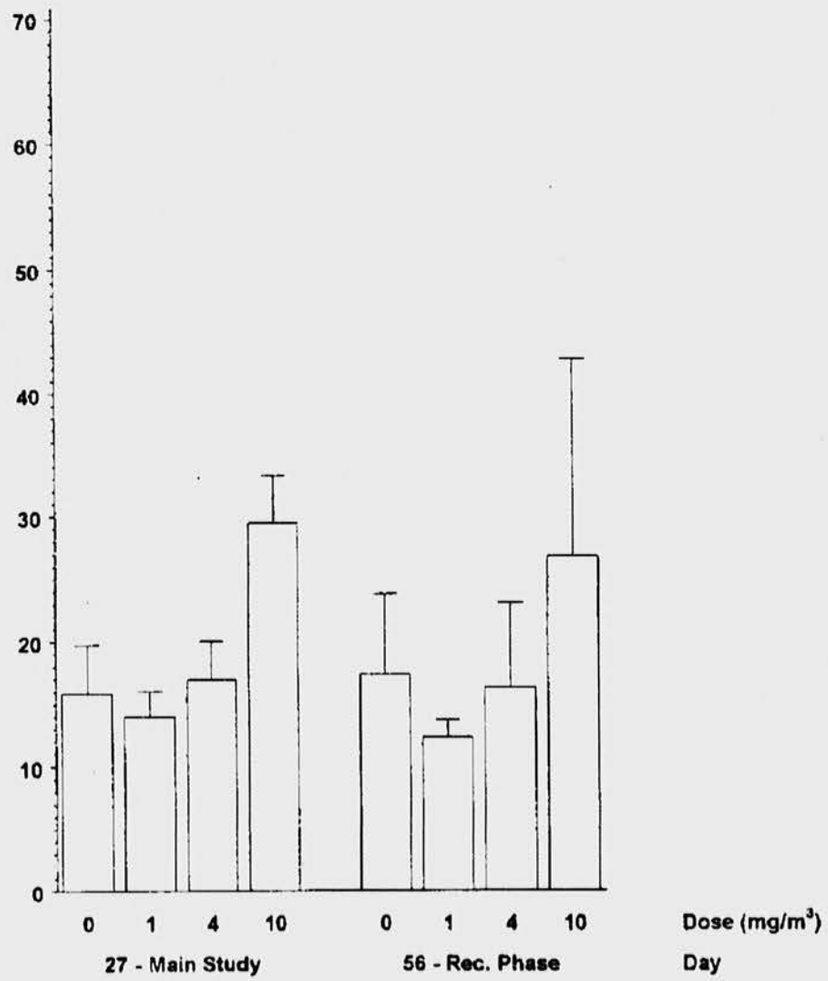


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN LACTATE DEHYDROGENASE (IU/l)

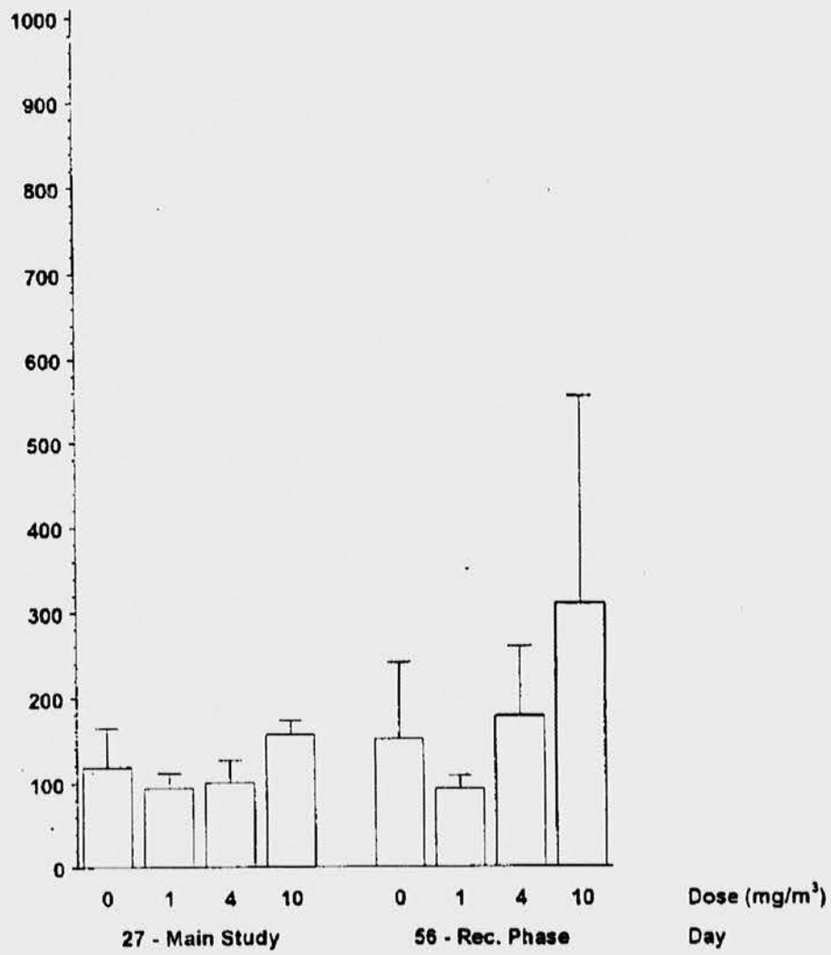


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN ALKALINE PHOSPHATASE (IU/l)

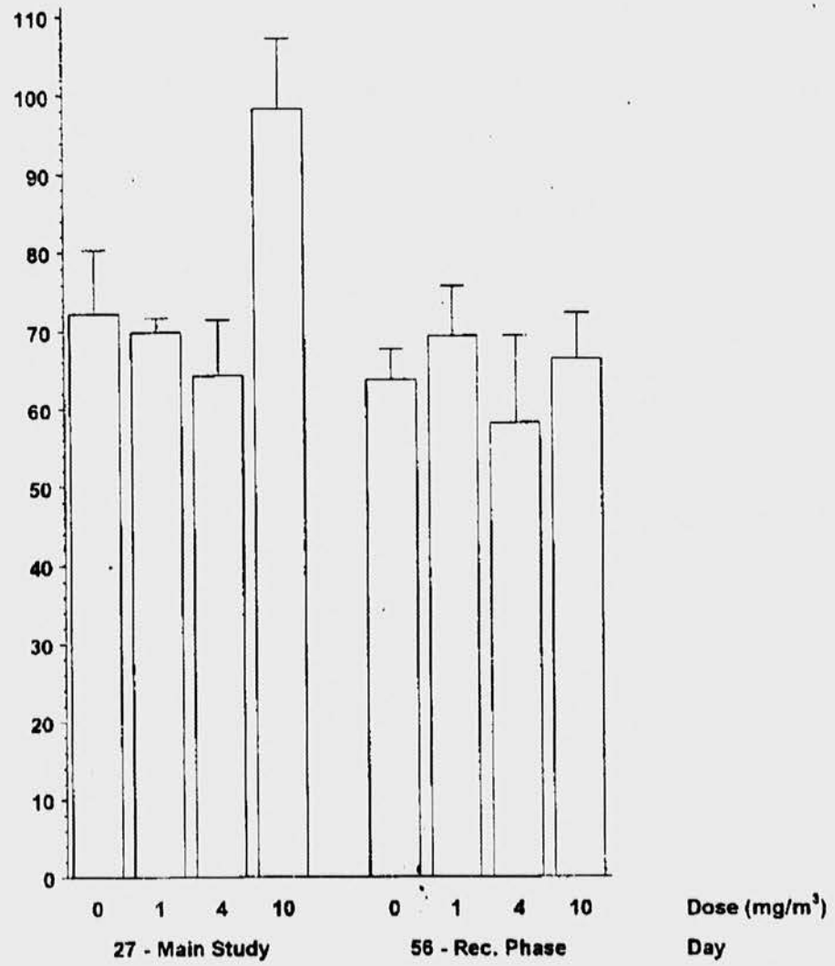


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN N-ACETYL GLUCOSAMINIDASE (IU/l)

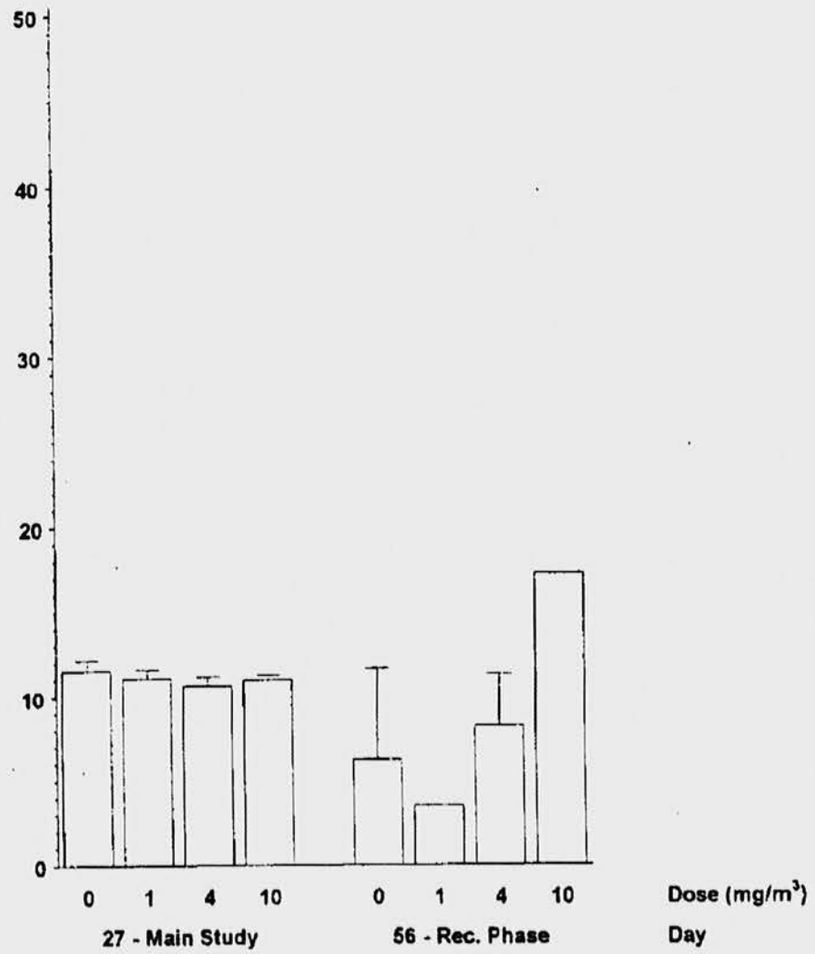


FIGURE 6 - LUNG LAVAGE
GROUP MEAN LAVAGE SUPERNATANT PHOSPHOLIPID CONCENTRATION (mmol/l)

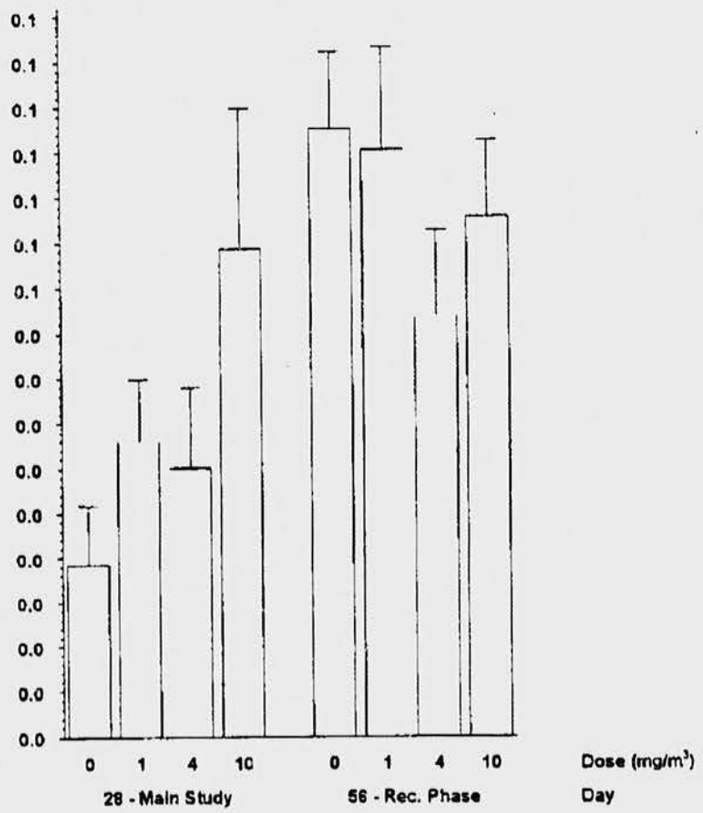


FIGURE 6 - LUNG LAVAGE
GROUP MEAN LAVAGE CELL PELLET PHOSPHOLIPID CONCENTRATION

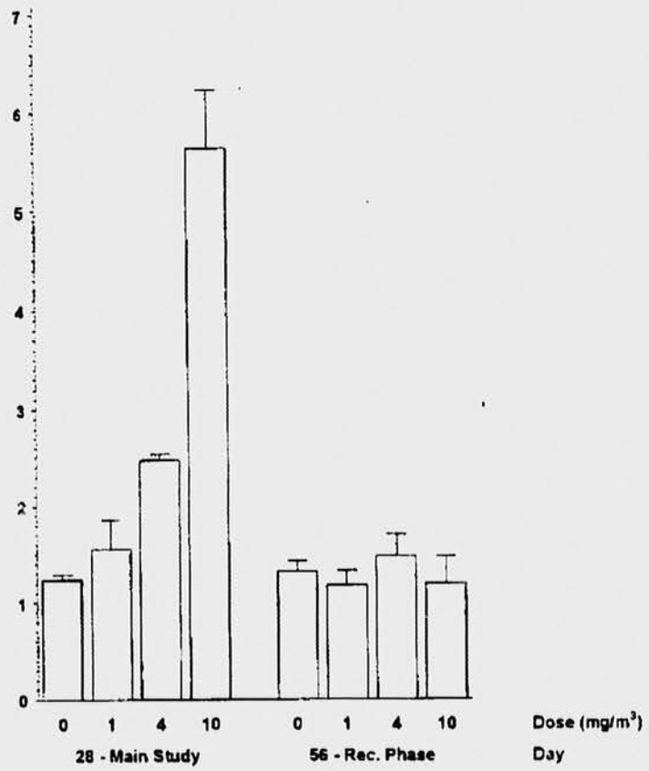


FIGURE 7 - GROUP MEAN LUNG WEIGHT (g)

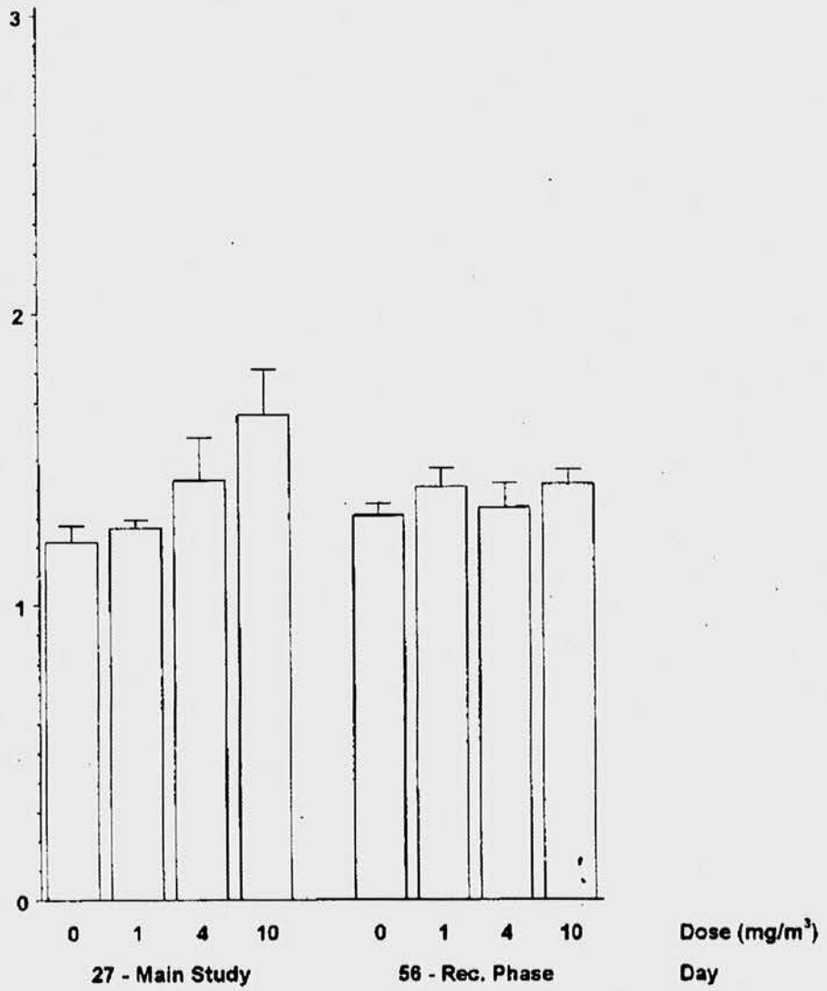


FIGURE 8 - BRDU LABELLING INDICES
GROUP MEAN TERMINAL BRONCHIOLES (%)

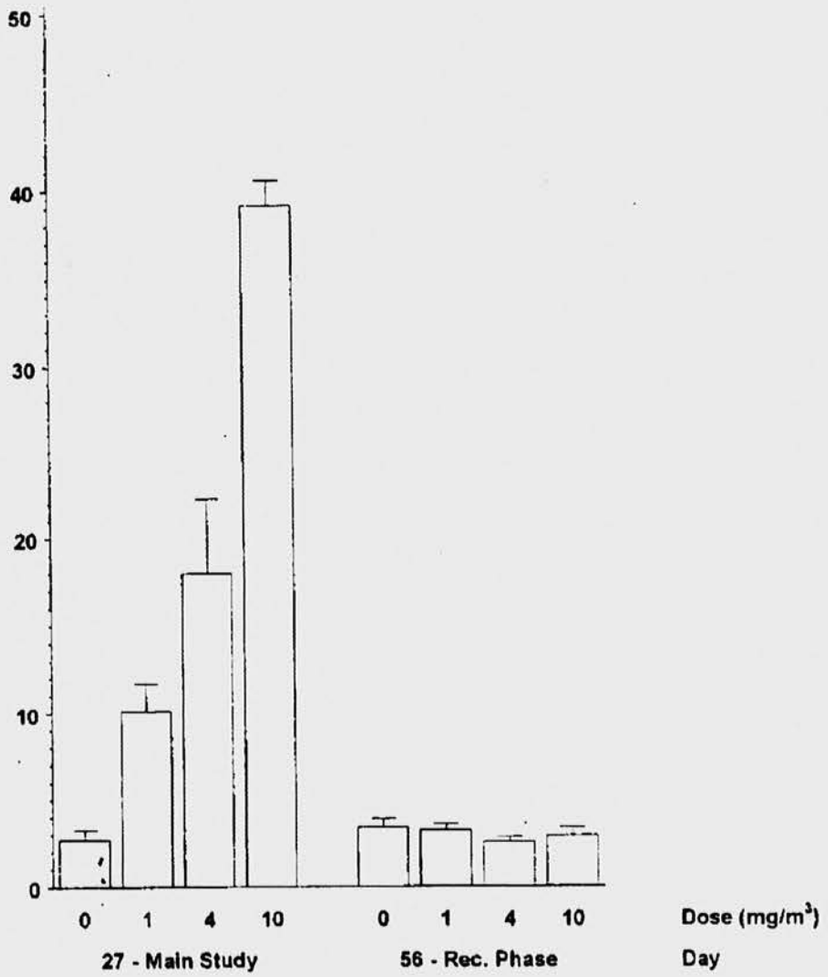


FIGURE 8 - BRDU LABELLING INDICES
GROUP MEAN CENTRO-ACINAR ALVEOLI (%)

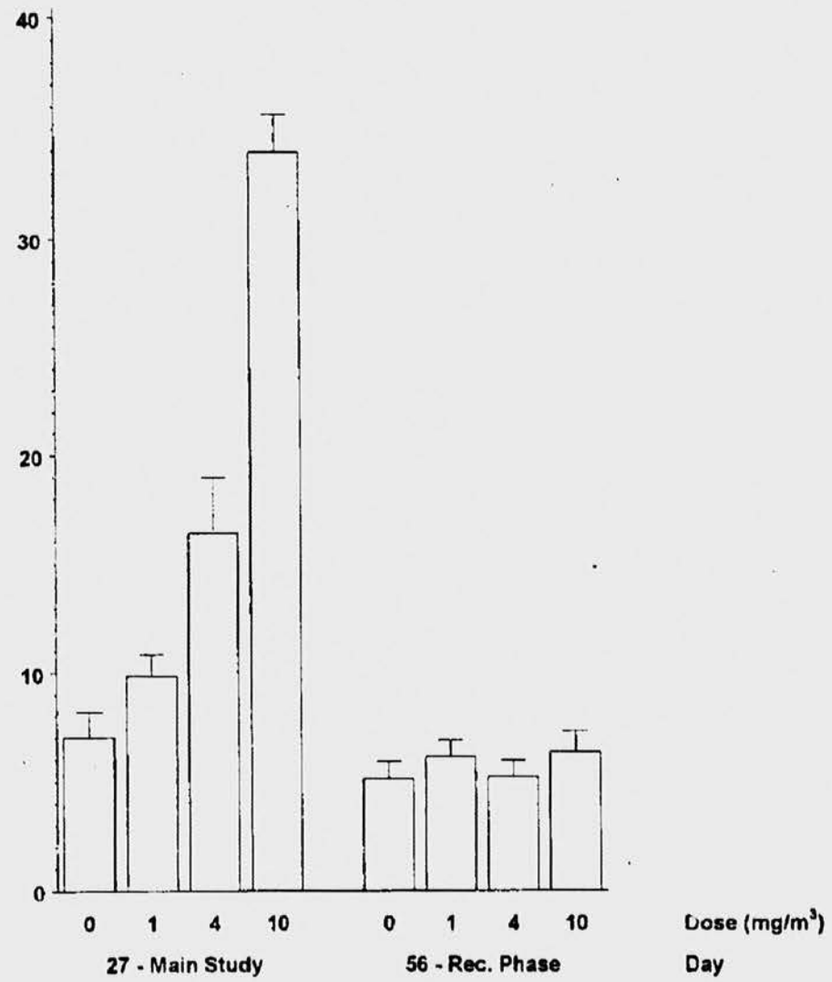


TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF DI-MDI GROUP 2

Exposure Date	Di-Mdi Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean	Di/Tri ratio
09-Sep-98	0.56	1.17	77.3	2.07
10-Sep-98	0.37	1.19	26.9	2.06
11-Sep-98	0.31	0.53	57.4	2.21
12-Sep-98	0.78	1.39	56.7	2.36
13-Sep-98	0.73	2.04	38.0	2.03
14-Sep-98	0.65	1.41	46.1	1.97
16-Sep-98	0.76	1.11	67.4	1.95
17-Sep-98	0.51	0.74	68.9	2.13
18-Sep-98	0.43	0.84	62.0	1.95
19-Sep-98	0.41	0.77	53.3	1.95
20-Sep-98	0.37	0.95	39.1	1.95
21-Sep-98	0.39	0.72	57.0	1.95
23-Sep-98	0.04	0.40	10.0	4.00
24-Sep-98	0.16	0.88	19.0	1.78
25-Sep-98	0.29	0.43	87.6	2.07
26-Sep-98	0.36	1.34	26.8	2.00
27-Sep-98	0.20	0.66	30.3	1.82
28-Sep-98	0.18	0.72	24.0	2.25
30-Sep-98	0.22	0.44	66.8	1.69
01-Oct-98	0.38	1.08	44.4	1.90
02-Oct-98	0.34	0.85	41.5	1.89
03-Oct-98	0.55	0.86	88.8	1.96
04-Oct-98	0.18	0.46	38.4	2.00
05-Oct-98	0.61	1.31	47.8	1.85
Mean	0.41	0.93	49.0	2.07
SD	0.20	0.39	21.0	0.44

SD = Standard Deviation

TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF TRI-MDI GROUP 2

Exposure Date	Tri-Mdi Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean
09-Sep-98	0.27	1.17	38.6
10-Sep-98	0.18	1.19	13.0
11-Sep-98	0.14	0.53	26.3
12-Sep-98	0.33	1.39	24.3
13-Sep-98	0.36	2.04	18.7
14-Sep-98	0.33	1.41	23.1
16-Sep-98	0.39	1.11	34.4
17-Sep-98	0.24	0.74	31.7
18-Sep-98	0.22	0.84	31.5
19-Sep-98	0.21	0.77	27.1
20-Sep-98	0.19	0.95	19.8
21-Sep-98	0.20	0.72	29.6
23-Sep-98	0.01	0.40	2.5
24-Sep-98	0.09	0.88	10.1
25-Sep-98	0.14	0.43	45.1
26-Sep-98	0.18	1.34	13.8
27-Sep-98	0.11	0.66	15.9
28-Sep-98	0.08	0.72	10.6
30-Sep-98	0.13	0.44	38.5
01-Oct-98	0.20	1.08	23.5
02-Oct-98	0.18	0.85	20.9
03-Oct-98	0.28	0.86	46.5
04-Oct-98	0.09	0.46	19.2
05-Oct-98	0.33	1.31	25.5
Mean	0.20	0.93	24.6
SD	0.10	0.39	11.1

SD = Standard Deviation

TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF DI-MDI GROUP 3

Exposure Date	Di-Mdi Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean	Di/Tri ratio
09-Sep-98	0.89	2.54	32.6	2.07
10-Sep-98	0.97	1.65	52.3	2.94
11-Sep-98	1.79	4.38	44.2	2.45
12-Sep-98	1.32	3.43	37.9	2.54
13-Sep-98	1.27	3.34	43.9	1.98
14-Sep-98	0.93	2.96	30.6	2.02
16-Sep-98	1.77	3.53	50.0	2.01
17-Sep-98	1.57	3.56	44.7	2.18
18-Sep-98	1.75	4.53	38.5	1.90
19-Sep-98	1.69	3.85	44.0	1.86
20-Sep-98	1.65	4.84	34.0	1.94
21-Sep-98	1.65	3.91	42.1	1.94
23-Sep-98	1.22	4.16	29.6	2.39
24-Sep-98	1.56	3.89	40.4	1.90
25-Sep-98	1.60	3.62	44.4	1.90
26-Sep-98	1.31	4.12	33.1	1.96
27-Sep-98	1.64	4.93	33.5	2.28
28-Sep-98	1.38	4.28	31.6	2.23
30-Sep-98	1.63	4.68	35.0	2.40
01-Oct-98	0.99	2.96	38.8	1.94
02-Oct-98	3.02	5.98	53.4	1.81
03-Oct-98	1.48	4.04	35.1	1.83
04-Oct-98	1.38	4.01	35.0	1.94
05-Oct-98	1.47	4.02	36.7	1.96
Mean	1.50	3.88	39.2	2.10
SD	0.42	0.87	6.8	0.28

SD = Standard Deviation

TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF TRI-MDI GROUP 3

Exposure Date	Tri-Mdi Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean
09-Sep-98	0.43	2.54	15.8
10-Sep-98	0.33	1.65	19.5
11-Sep-98	0.73	4.38	17.1
12-Sep-98	0.52	3.43	15.3
13-Sep-98	0.64	3.34	21.9
14-Sep-98	0.46	2.96	15.2
16-Sep-98	0.88	3.53	24.8
17-Sep-98	0.72	3.56	20.3
18-Sep-98	0.92	4.53	20.3
19-Sep-98	0.91	3.85	23.8
20-Sep-98	0.85	4.84	17.5
21-Sep-98	0.85	3.91	21.6
23-Sep-98	0.51	4.16	12.4
24-Sep-98	0.82	3.89	21.2
25-Sep-98	0.84	3.62	23.3
26-Sep-98	0.67	4.12	16.9
27-Sep-98	0.72	4.93	14.6
28-Sep-98	0.62	4.28	14.4
30-Sep-98	0.68	4.68	14.2
01-Oct-98	0.51	2.96	20.3
02-Oct-98	1.67	5.98	29.6
03-Oct-98	0.81	4.04	19.0
04-Oct-98	0.71	4.01	18.0
05-Oct-98	0.75	4.02	18.8
Mean	0.73	3.88	19.0
SD	0.26	0.87	4.0

S D = Standard Deviation

TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF DI-MDI GROUP 4

Exposure Date	Di-MDI Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean	Di/Tri ratio
09-Sep-98	4.36	10.40	41.8	2.06
10-Sep-98	2.91	7.05	41.7	2.37
11-Sep-98	1.41	4.95	40.1	2.43
12-Sep-98	4.90	13.70	35.8	2.07
13-Sep-98	3.21	7.48	42.8	2.07
14-Sep-98	5.61	11.89	47.0	1.90
16-Sep-98	5.30	9.52	55.4	2.11
17-Sep-98	4.28	8.81	48.5	2.29
18-Sep-98	4.14	11.38	35.8	2.05
19-Sep-98	4.22	10.34	41.4	1.96
20-Sep-98	3.16	7.47	42.3	2.16
21-Sep-98	4.09	8.63	48.9	2.06
23-Sep-98	4.69	11.53	40.7	1.99
24-Sep-98	4.05	9.45	43.2	2.26
25-Sep-98	4.56	12.58	36.4	2.53
26-Sep-98	3.34	8.30	44.0	2.15
27-Sep-98	4.05	12.52	32.8	2.93
28-Sep-98	1.73	13.01	13.3	2.93
30-Sep-98	2.33	8.34	27.9	2.68
01-Oct-98	2.29	12.26	19.2	2.66
02-Oct-98	4.17	12.18	34.9	2.11
03-Oct-98	5.62	13.58	41.3	1.97
04-Oct-98	4.84	10.59	47.3	2.15
05-Oct-98	2.91	11.24	28.9	2.09
Mean	3.84	10.30	38.4	2.25
SD	1.15	2.31	9.8	0.30

SD = Standard Deviation

TABLE 1 - ATMOSPHERIC CONCENTRATIONS OF TRI-MDI GROUP 4

Exposure Date	Tri-Mdi Analysed Concentration Daily Mean(mg/m3)	Polymeric MDI Gravimetric Concentration Daily Mean(mg/m3)	Percent Total Particulate Daily Mean
09-Sep-98	2.12	10.40	20.4
10-Sep-98	1.23	7.05	17.4
11-Sep-98	0.58	4.95	16.9
12-Sep-98	2.37	13.70	17.3
13-Sep-98	1.55	7.48	20.8
14-Sep-98	2.95	11.89	24.7
16-Sep-98	2.51	9.52	26.2
17-Sep-98	1.87	8.81	21.1
18-Sep-98	2.02	11.38	17.3
19-Sep-98	2.15	10.34	21.1
20-Sep-98	1.46	7.47	19.5
21-Sep-98	1.99	8.63	23.6
23-Sep-98	2.36	11.53	20.5
24-Sep-98	1.79	9.45	19.3
25-Sep-98	1.80	12.58	14.7
26-Sep-98	1.55	8.30	21.1
27-Sep-98	1.38	12.52	11.4
28-Sep-98	0.59	13.01	4.6
30-Sep-98	0.87	8.34	10.4
01-Oct-98	0.86	12.26	7.0
02-Oct-98	1.98	12.21	16.6
03-Oct-98	2.85	13.58	20.9
04-Oct-98	2.25	10.59	22.1
05-Oct-98	1.39	11.24	14.1
Mean	1.77	10.30	17.9
SD	0.65	2.31	5.3

SD =_ Standard Deviation

TABLE 2 - AERODYNAMIC PARTICLE SIZE DISTRIBUTION

Day No	Group 2		Group 3		Group 4	
	D_{50}	GSD	D_{50}	GSD	D_{50}	GSD
1					0.96	1.51
2					0.81	1.55
3	1.32	3.08	1.41	2.01	1.23	1.80
4	0.85	1.84	1.43	1.76	1.37	1.46
5	0.93	1.85	1.80	2.37	1.03	1.46
6	0.68	1.92	1.52	1.49	1.27	1.52
7	1.25	3.58				
8			1.79	1.78		
9					2.11	1.51
14	1.48	2.97	2.05	2.79		
15					0.53	1.89
19					0.73	2.63
20					0.86	1.50
21	1.47	2.08				
22			1.26	1.47		

GSD - geometric standard deviation

D_{50} - mass median aerodynamic diameter

GLOSSARY FOR TABLE 3

Key:-

The following abbreviations may appear in this Table.

NO. OF OBS	-	Number of observations. This may represent the recording of an observation on more than one occasion during the day for any individual animal.
NO	-	number
SEE FREE TEXT	-	free text comments are displayed in the Individual animal data supplement

TABLE 3 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS

SEX: FEMALE	0 mg/m3	0.93 mg/m3	3.88 mg/m3	10.30 mg/m3
COLD				
NO. OF OBS.				1
NO. OF ANIMALS				1
DAYS FROM - TO				26 - 26
HUNCHED				
NO. OF OBS.				2
NO. OF ANIMALS				1
DAYS FROM - TO				26 - 27
KILLED TERMINATION				
NO. OF OBS.	40	30	30	40
NO. OF ANIMALS	40	30	30	40
DAYS FROM - TO	27 - 56	27 - 56	27 - 56	27 - 56
MALOCCLUSION				
NO. OF OBS.				6
NO. OF ANIMALS				1
DAYS FROM - TO				8 - 13
PALE				
NO. OF OBS.				2
NO. OF ANIMALS				1
DAYS FROM - TO				26 - 27
PILOERECTION				
NO. OF OBS.				2
NO. OF ANIMALS				1
DAYS FROM - TO				26 - 27
SEE FREE TEXT				
NO. OF OBS.				6
NO. OF ANIMALS				1
DAYS FROM - TO				8 - 13

GLOSSARY FOR STATISTICAL TABLES 4-7

Key to results of statistical tests:-

** Statistically significant difference from the control group mean at the 1% level (Student's t-test, two-sided).

* Statistically significant difference from the control group mean at the 5% level (Student's t-test, two-sided).

- **Key:**
- SD - standard deviation
- N - number of animals analysed

Adjusted mean:

Group mean bodyweight corrected for intergroup differences in group mean initial bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup difference in group mean initial bodyweight and the strength of the relationship between initial bodyweight and subsequent bodyweights.

Organ weight adjusted for bodyweight:

Group mean organ weights corrected for intergroup differences in group mean terminal bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup difference in group mean terminal bodyweight and the strength of the relationship between organ weight and terminal bodyweight (Shirley 1996).

GLOSSARY FOR STATISTICAL TABLES 4-7

Data omitted from the statistical analysis

For n-acetyl glucosaminidase all missing values were recorded as below the limit of detection (3). The omission of these values has not affected the interpretation.

Lung - female 149, day 56.

TABLE 4 - INTERGROUP COMPARISON OF BODYWEIGHTS (g)

MAIN STUDY

			Concentration (mg/m^3) 0(Control)	0.93	5.88	10.3
Day 1		MEAN	232.4	233.5	236.2	235.0
		S.D.	11.6	9.1	15.9	12.6
		N	20	15	15	20
Day 8		MEAN	244.4	239.0	243.1	244.4
		S.D.	11.1	10.4	11.5	13.2
		N	20	15	15	20
	ADJUSTED	MEAN	245.8	239.8*	241.8	243.8
Day 15		MEAN	250.7	246.1	250.9	253.3
		S.D.	10.7	11.1	11.8	11.4
		N	20	15	15	20
	ADJUSTED	MEAN	252.0	247.0*	249.8	252.7
Day 22		MEAN	263.3	259.4	263.3	265.8
		S.D.	12.8	14.3	16.0	13.3
		N	20	15	15	20
	ADJUSTED	MEAN	264.8	259.9	261.6	265.1
Day 27		MEAN	258.9	258.1	264.2	260.6
		S.D.	14.2	13.8	15.1	14.3
		N	20	15	15	20
	ADJUSTED	MEAN	260.4	257.8	261.5	259.9

POLYMERIC MDI: 28 DAY REPEAT EXPOSURE STUDY IN FEMALE RATS (6 HOURS PER DAY 5 DAYS PER WEEK) AND POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

TABLE 4 - INTERGROUP COMPARISON OF BODYWEIGHTS (g) RECOVERY PHASE

		Concentration (mg/m^3)			
		0(Control)	0.93	3.88	10.3
Day 1	MEAN	228.6	230.6	233.9	231.0
	S.D.	9.6	11.1	11.5	13.9
	N	20	15	15	20
Day 8	MEAN	236.5	237.4	241.8	235.0
	S.D.	10.0	13.6	10.4	14.5
	N	20	15	15	20
	ADJUSTED MEAN	238.4	237.9	239.4	234.8
Day 15	MEAN	246.8	244.3	250.3	244.5
	S.D.	12.5	16.5	9.4	15.4
	N	20	15	15	20
	ADJUSTED MEAN	249.0	245.1	247.9	244.4
Day 22	MEAN	255.5	253.3	263.2	252.9
	S.D.	14.8	14.3	9.0	15.7
	N	20	15	15	20
	ADJUSTED MEAN	257.7	253.6	260.3	252.7
Day 29	MEAN	258.3	258.1	265.0	252.2
	S.D.	14.4	15.4	12.0	17.0
	N	20	15	15	20
	ADJUSTED MEAN	260.6	259.1	262.7	252.0**
Day 36	MEAN	270.4	269.3	275.3	272.4
	S.D.	16.1	14.6	11.5	15.9
	N	20	15	15	20
	ADJUSTED MEAN	272.4	270.7	273.8	272.3
Day 43	MEAN	274.4	273.5	282.4	280.9
	S.D.	16.3	17.1	12.9	14.3
	N	20	15	15	20
	ADJUSTED MEAN	276.2	275.2	281.4	280.7
Day 50	MEAN	282.7	280.8	292.5	289.0
	S.D.	17.3	18.3	13.8	15.9
	N	20	15	15	20
	ADJUSTED MEAN	284.9	281.2	289.7	288.9
Day 56	MEAN	286.4	281.3	293.9	292.5
	S.D.	17.6	22.6	14.7	15.2
	N	20	15	15	20
	ADJUSTED MEAN	288.6	281.3	290.7	292.3

POLYMERIC MDI: 28 DAY REPEAT EXPOSURE STUDY IN FEMALE RATS (6 HOURS PER DAY 5 DA . S PER WEEK) AND POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

TABLE 5 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

MAIN STUDY

		Concentration (mg/m³)			
		0(Control)	0.93	3.88	10.3
Total Cell Count (x10**6/ml)					
	MEAN	3.46	3.48	5.70	9.68**
	S.D.	0.52	0.61	2.90	3.33
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	3.44	3.44	5.47	8.15**
	S.D.	0.52	0.60	2.82	3.35
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.008	0.017	0.073**	0.682**
	S.D.	0.008	0.011	0.066	0.473
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.010	0.022	0.156**	0.845**
	S.D.	0.003	0.017	0.113	0.557
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	15.8	14.0	16.9	29.4**
	S.D.	7.7	3.9	6.2	7.6
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	117.6	93.4	100.2	156.0
	S.D.	91.7	33.9	50.5	32.5
	N	5	5	5	5
Alkaline Phosphatase (IU/l)					
	MEAN	72.2	69.8	64.2	98.2**
	S.D.	15.9	3.6	14.1	17.5
	N	5	5	5	5
N-acetyl glucosaminidase (IU/l)					
	MEAN	11.52	11.07	10.62	10.96
	S.D.	1.30	1.07	1.15	0.66
	N	5	5	5	5
Lavage Supernatant Phospholipid Concentration					
	MEAN	0.04	0.07	0.06	0.11*
	S.D.	0.03	0.02	0.03	0.06
	N	5	4	5	5
Lavage Cell Pellet Phospholipid Concentration					
	MEAN	1.24	1.55	2.47**	5.64**
	S.D.	0.12	0.60	0.13	1.18
	N	5	5	5	5

POLYMERIC MDI: 28 DAY REPEAT EXPOSURE STUDY IN FEMALE RATS (6 HOURS PER DAY 5 DAYS PER WEEK) AND POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

TABLE 5 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID RECOVERY PHASE

		Concentration (mg/m³)			
		0(Control)	0.93	3.88	10.3
Total Cell Count (x10**6/ml)					
	MEAN	2.90	2.26	1.90*	2.08
	S.D.	0.71	0.81	0.29	0.72
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	2.88	2.25	1.89*	2.07
	S.D.	0.70	0.80	0.29	0.72
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.015	0.005	0.006	0.002**
	S.D.	0.010	0.004	0.007	0.002
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.006	0.008	0.007	0.004
	S.D.	0.005	0.006	0.003	0.001
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	17.3	12.3	16.2	26.7
	S.D.	12.7	2.8	13.4	31.4
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	150.6	92.2	177.2	308.4
	S.D.	175.2	30.1	160.2	482.8
	N	5	5	5	5
Alkaline Phosphatase (IU/l)					
	MEAN	63.6	69.2	58.0	66.2
	S.D.	7.8	12.6	22.0	11.6
	N	5	5	5	5
N-Acetyl glucosaminidase (IU/l)					
	MEAN	6.21	3.48	8.22	17.20
	S.D.	4.20		2.35	
	N	2	1	2	1
Lavage Supernatant Phospholipid Concentration					
	MEAN	0.14	0.13	0.09	0.12
	S.D.	0.03	0.04	0.04	0.03
	N	5	5	5	5
Lavage Cell Pellet Phospholipid Concentration					
	MEAN	1.32	1.17	1.47	1.19
	S.D.	0.20	0.31	0.45	0.55
	N	5	5	5	5

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS (g)

MAIN STUDY

		Concentration (mg/m^3)			
		0(Control)	0.93	3.88	10.3
Terminal Bodyweight (g)					
	MEAN	269.4	261.0	274.4	266.6
	S.D.	17.5	10.9	12.8	10.7
	N	5	5	5	5
Organ Weight (g)					
	MEAN	1.22	1.26	1.43	1.65**
	S.D.	0.11	0.06	0.29	0.31
	N	5	5	5	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.45	0.48	0.52	0.62
	S.D.	0.04	0.02	0.08	0.10
	N	5	5	5	5
Organ Weight Adjusted for Bodyweight		1.20	1.33	1.37	1.66**

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS RECOVERY PHASE

		Concentration (mg/m³)			
		0(Control)	0.93	3.88	10.3
Terminal Bodyweight (g)					
	MEAN	288.6	303.0	292.5	298.6
	S.D.	21.4	19.3	19.3	10.9
	N	5	5	4	5
Organ Weight (g)					
	MEAN	1.31	1.40	1.33	1.41
	S.D.	0.08	0.13	0.14	0.10
	N	5	5	4	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.45	0.46	0.45	0.47
	S.D.	0.02	0.02	0.02	0.03
	N	5	5	4	5
Organ Weight Adjusted For Bodyweight		1.34	1.36	1.35	1.40

TABLE 7 - INTERGROUP COMPARISON OF BRDU LABELLING INDICES MAIN STUDY

		Concentration (mg/m^3) 0 (Control)	0.93	3.88	10.3
Terminal Bronchioles (%)					
	MEAN	2.7	10.1**	17.9**	39.1**
	S.D.	1.1	3.2	8.5	2.9
	N	5	5	5	5
Centro-acinar alveoli (%)					
	MEAN	7.0	9.8	16.4**	33.9**
	S.D.	2.3	2.0	5.0	3.4
	N	5	5	5	5
Terminal Bronchioles + Centro-Acinar alveoli (%)					
	MEAN	4.9	10.0**	17.2**	36.5**
	S.D.	1.2	2.1	6.5	2.7
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF BRDU LABELLING INDICES RECOVERY PHASE

		Concentration (mg/m³)			
		0(Control)	0.93	3.88	10.3
Terminal Bronchioles (%)					
	MEAN	3.4	3.2	2.5	2.9
	S.D.	1.0	0.7	0.6	1.0
	N	5	5	5	5
Centro-acinar alveoli (%)					
	MEAN	5.1	6.1	5.2	6.3
	S.D.	1.5	1.5	1.4	1.9
	N	5	5	5	5
Terminal Bronchioles + Centro-Acinar alveoli (%)					
	MEAN	4.2	4.7	3.9	4.6
	S.D.	1.2	0.8	0.8	1.1
	N	5	5	5	5

TABLE 8 - INTERGROUP COMPARISON OF MICROSCOPIC FINDINGS INCIDENCE MAIN STUDY

NON-NEOPLASTIC MORPHOLOGIES SEX: FEMALES	0 mg/m3	0.93 mg/m3	3.88 mg/m3	10.30 mg/m3
FEMALES ON STUDY	40	30	30	40
ANIMALS COMPLETED	5	5	5	5
LUNG				
EXAMINED	5	5	5	5
NO ABNORMALITIES DETECTED	5	4	3	0
Increased perivascular neutrophil infiltration (TOTAL)	0	0	1	0
slight	0	0	1	0
Interstitial thickening - central acinar region (TOTAL)	0	0	1	5
minimal	0	0	1	0
slight	0	0	0	5
Bronchiolitis - terminal region (TOTAL)	0	1	1	5
minimal	0	1	1	2
slight	0	0	0	2
moderate	0	0	0	1
Pigmented alveolar macrophages - accumulation (TOTAL)	0	0	0	5
slight	0	0	0	2
moderate	0	0	0	3
Hypertrophy and hyperplasia - terminal bronchiole (TOTAL)	0	0	0	5
slight	0	0	0	4
moderate	0	0	0	1

TABLE 8 - INTERGROUP COMPARISON OF MICROSCOPIC FINDINGS INCIDENCE RECOVERY PHASE

NON-NEOPLASTIC MORPHOLOGIES SEX: FEMALES	0 mg/m3	0.93 mg/m3	3.88 mg/m3	10.30 mg/m3
FEMALES ON STUDY	40	30	30	40
ANIMALS COMPLETED	5	5	5	5
LUNG				
EXAMINED	5	5	5	5
NO ABNORMALITIES DETECTED	5	5	5	0
Interstitial thickening - central acinar region (TOTAL)	0	0	0	1
slight	0	0	0	1
Bronchiolitis - terminal region (TOTAL)	0	0	0	1
minimal	0	0	0	1
Pigmented alveolar macrophages - accumulation (TOTAL)	0	0	0	5
minimal	0	0	0	3
slight	0	0	0	2

TABLE 9 - INTERGROUP COMPARISON OF LUNG LAVAGE CELL PATHOLOGY

Dose (mg/m^3) **Main study**	0	0.93	3.88	10.3
Foamy macrophages				
minimal	4	5	0	0
slight	1	0	4	0
moderate	0	0	1	0
marked	0	0	0	5
Recovery phase				
Foamy macrophages				
minimal	5	4	4	2
slight	0	1	1	3
moderate	0	0	0	0
marked	0	0	0	0

TABLE 10 - INTERGROUP COMPARISON OF ELECTRON MICROSCOPY MAIN STUDY

	0mg/m³	0.93mg/m³	3.88mg/m³	10.3mg/m³
LUNG				
EXAMINED	5	5	5	5
NAD	5			
INTERSTITIAL THICKENING	0	0	0	3
ALVEOLAR TYPE II CELLS				
Lamellar bodies				
increase in number				
minimal	0	1	0	1
increase in size				
minimal	0	1	2	3
MACROPHAGES				
Lamellar surfactant				
minimal	0	0	1	1
slight	0	0	0	1
Crystalline surfactant				
minimal	0	0	1	0
Amorphous surfactant				
minimal	0	3	0	1
slight	0	0	0	0
moderate	0	0	0	1

TABLE 10 - INTERGROUP COMPARISON OF ELECTRON MICROSCOPY

MAIN STUDY

	0mg/m³	0.93mg/m³	3.88mg/m³	10.3mg/m³
LUMEN				
Crystalline surfactant				
minimal	0	1	1	1
slight	0	0	0	1
Debris				
minimal	0	0	2	2
slight	0	0	0	0
moderate	0	0	1	1
Lamella surfactant				
minimal	0	1	1	3
slight	0	0	0	0
moderate	0	0	1	1

TABLE 10 - INTERGROUP COMPARISON OF ELECTRON MICROSCOPY

RECOVERY PHASE

	0mg/m³	0.93mg/m³	3.88mg/m³	10.3mg/m³
LUNG				
EXAMINED	5	5	5	5
ANIMALS NAD	5	0	0	0
INTERSTITIAL THICKENING	0	0	0	1
ALVEOLAR TYPE II CELLS				
Lamellar bodies				
increase in number				
minimal	0	0	2	1
increase in size				
minimal	0	0	0	0
slight	0	0	0	0
MACROPHAGES				
Lamellar surfactant				
minimal	0	0	0	0
slight	0	0	0	0
Crystalline surfactant				
minimal	0	1	0	1
Amorphous surfactant				
minimal	0	2	2	1

TABLE 10 - INTERGROUP COMPARISON OF ELECTRON MICROSCOPY

RECOVERY PHASE

	0mg/m³	0.93mg/m³	3.88mg/m³	10.3mg/m³
LUMEN				
Crystalline surfactant				
minimal	0	2	1	2
Debris				
minimal	0	1	1	1
slight	0	0	0	1
Lamella surfactant				
minimal	0	3	1	1
slight	0	0	0	0

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE DETERMINATION

METHOD SUMMARY

The test substance was extracted/desorbed from filters with a known volume of acetic anhydride in acetonitrile. After appropriate derivatisation and dilution an aliquot was removed. The samples and standards were then analysed by High Performance Liquid Chromatography (HPLC).

CHEMICALS AND REAGENTS

Acetonitrile, HPLC grade

Water, Milli Q+ grade (Millipore)

Glacial acetic acid, analytical grade

Sodium acetate, analytical grade

Toluene, HPLC grade

Acetic anhydride, analytical grade

Diethyl phthalate, analytical grade

1-(2-methoxyphenyl)piperazine, analytical grade

DERIVATISATION

Derivatising Solution

Isocyanate samples were derivatised using 1-(2-methoxyphenyl)piperazine (1,2-MP).

Approximately 400mg of 1,2-MP was accurately weighed into a 10ml volumetric flask and 500mg diethyl phthalate added. This was made up to volume with toluene and designated Solution A, for use in coating filters.

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE DETERMINATION Filter preparation

Solution A (2ml) and toluene (5.25ml) were mixed and 200µl of the resultant solution was used to coat each filter. The filters were placed in the dark to dry and sealed in a vials for storage. These dry filters were then supplied to the Inhalation Group.

Desorbing Solution

Approximately 50mg of 1,2-MP was accurately weighed into a 100ml volumetric flask and made to volume with toluene to produce a stock 1,2-MP solution. This stock solution was diluted to make 260µmolar solution which was used to complete the derivatisation of the filters.

CALIBRATION STANDARDS

Analytical standard

Suprasec 5005, CTL Reference Y00122/021, with an assumed purity of 44.11% w/w Di-MDI and 22.94% w/w Tri-MDI was used.

Varying amounts of Suprasec 5005 was accurately weighed into a volumetric flask and diluted to volume with toluene to give a stock standard of known concentration. (e.g. 157.5mg Suprasec 5005 made to 100ml with toluene to give a stock solution then diluted to give working standards, after derivatisation.)

Further appropriate dilutions were made with toluene in volumetric flasks to give a range of solutions, within 3.645µg/ml to 24.525µg/ml Di-MDI and 1.89µg/ml to 12.750µg/ml Tri-MDI.

The purity of the test substance was taken into account in the preparation of the standard solutions.

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE DETERMINATION

PROCEDURE

Sample preparation

A known volume (2ml) of 260µmolar 1,2-MP solution was added to each filter in the container provided with the sample. The vial was sealed and left for 24 hours in darkness. Acetic anhydride (100µl) was then added to the vial and the whole sample was evaporated to dryness under nitrogen.

A known volume of acetonitrile (5/10ml) was added and gently agitated in the vial. The filter and solution were then decanted into UNIPREP syringeless filter and filtered into a clean vial. An aliquot was then removed for subsequent analysis.

High Performance Liquid Chromatography Conditions

Modular system	:	LC Module 1 (Waters)	
Comprising			
Pump	:	600 Series (Waters)	
Autosampler	:	717 autosampler (Waters	
Detectors	:	484 UV detector (Waters)	
	:	464 electrochemical detector (Waters)	
Mobile phase	:	acetonitrile (60% v/v)	
		0.5% sodium acetate buffer pH 6 (40% v/v)	
Flow rate	:	1ml/min	
Detector wavelength	:	242nm	
464 Electrochemical			
detector settings	:	Detector mode	: DC
	:	Working electrode	: Glassy carbon
	:	Reference electrode	: Ag / AgCl
	:	Potential	: +800mV

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE DETERMINATION

Current range		0.2µA
Column	:	25cm x 4.6mm ID S5ODS2 (HiChrom)
Injection volume	:	25µl
Data handling	:	Waters 860 Data System (Waters)

CALIBRATION

The analysis system was calibrated using a range of standards to determine the linearity of response.

CALCULATION OF RESULTS

Total Particulate Samples

Analysed atmosphere concentration (mg/m^3) $Ca = \frac{Cs \times Df}{Va \times 1000}$

Where	Cs	=	Calculated sample concentration from data system (µg/ml)
	Df	=	Dilution factor
	Va	=	Atmosphere sample volume (l)
		=	Sample collection time (min) x flow (l/min)

$$\% \text{ Total Particulate} = \frac{Ca \times 100}{Cg}$$

Where Cg = Gravimetric atmosphere concentration

LIMIT OF DETECTION

The limit of detection was calculated to be approximately 0.3µg/ml Di-MDI and 0.6µg/ml Tri-MDI in the analysed solution, corresponding to an atmosphere concentration of 0.01mg/m^3 Di-MDI and 0.02mg/m^3 Tri-MDI.

APPENDIX B - PARTICLE SIZE CLASSIFICATION

1. Mass median aerodynamic diameter (MMAD)

The mass median aerodynamic diameter (MMAD) of an aerosol, or part of an aerosol, is the diameter of a unit density sphere having the same terminal settling velocity as a particle shown to divide the size distribution of the aerosol in half when measured by mass. The algebraic symbol commonly used for the MMAD is "D_{50}".

2. Geometric standard deviation (GSD)

The GSD of an aerosol is the ratio of the mean of the distribution to the mean ± 1 standard deviation i.e.:

$$GSD\ (\delta g) = \frac{D50}{D16} = \frac{D84}{D50} = \sqrt{\frac{D84}{D16}}$$

This relationship is only valid for aerosols with a log normal distribution, which is considered to be the case in this study.

APPENDIX C - ALLOCATION OF RATS TO EXPERIMENTAL GROUPS

The animals were distributed amongst experimental groups by a method designed to ensure that any unhealthy rats or rats at the extremes of the weight range were excluded.

The weights were recorded and sorted using a computer sort feature. All the stock animals were weighed, clinical observations noted and the data recorded directly electronically. The weights were then sorted electronically into order with the highest weight listed first, followed by the next highest etc. Animals at extremes of the weight range, or showing adverse clinical changes, were discarded.

A Latin Square was generated and was used for the allocation of animals to the experimental groups. The heaviest animal was allocated the lowest number in the group of replicate denoted by the number on the Latin Square and ear-punched with the lowest available experimental number for that cage shown on the rack plan. This procedure was carried out until all cages in each replicate contained one rat.

The procedure was then repeated until each cage contained five rats.

APPENDIX D - ARRANGEMENT OF ANIMALS ON THE RACKS SHOWING INDIVIDUAL ANIMAL NUMBERS AND POSITIONS

Females Rack 1

Rep 1	1-5 (1)	21-25 (2)	51-55 (4)	36-40 (3)
Rep 2	26-30 (2)	56-60 (4)	41-45 (3)	6-10 (1)
Rep 3	61-65 (4)	NA (3)	11-15 (1)	NA (2)
Rep 4	46-50 (3)	16-20 (1)	31-35 (2)	66-70 (4)

Females Rack 2

Rep 5	136-140 (3)	101-105 (1)	151-155 (4)	121-125 (2)
Rep 6	126-130 (2)	156-160 (4)	106-110 (1)	141-145 (3)
Rep 7	111-115 (1)	NA (2)	NA (3)	161-165 (4)
Rep 8	166-170 (4)	146-150 (3)	131-135 (2)	116-120 (1)

Group numbers are given in parentheses

NA no animals allocated to these groups

APPENDIX E - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

1. Purpose

The nose-only method of exposure was used as the purpose of the study was to investigate the effect on the lungs of exposure to an aerosol of the test material.

2. Design (Figure 1)

2.1 The chamber

The chamber consisted of sections of PERSPEX tubing (6mm wall thickness) with an internal diameter of 28cm and height of 15cm (approximate volume 9.2 litres). Each section was drilled with ten equidistant holes of 28mm diameter into which the restraining tubes were pushed to give a good seal. There was also one sampling port.

The chamber located on to a base-plate, fitted with castors for manoeuvrability. A conical aluminium lid ensured good distribution of the atmosphere across the chamber, the atmosphere having being generated from above. The conical lid and the base together had a volume of approximately 9.2 litres. In this study four sections were connected, giving a total volume of approximately 46 litres.

APPENDIX E - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

2.2 The restraining tubes

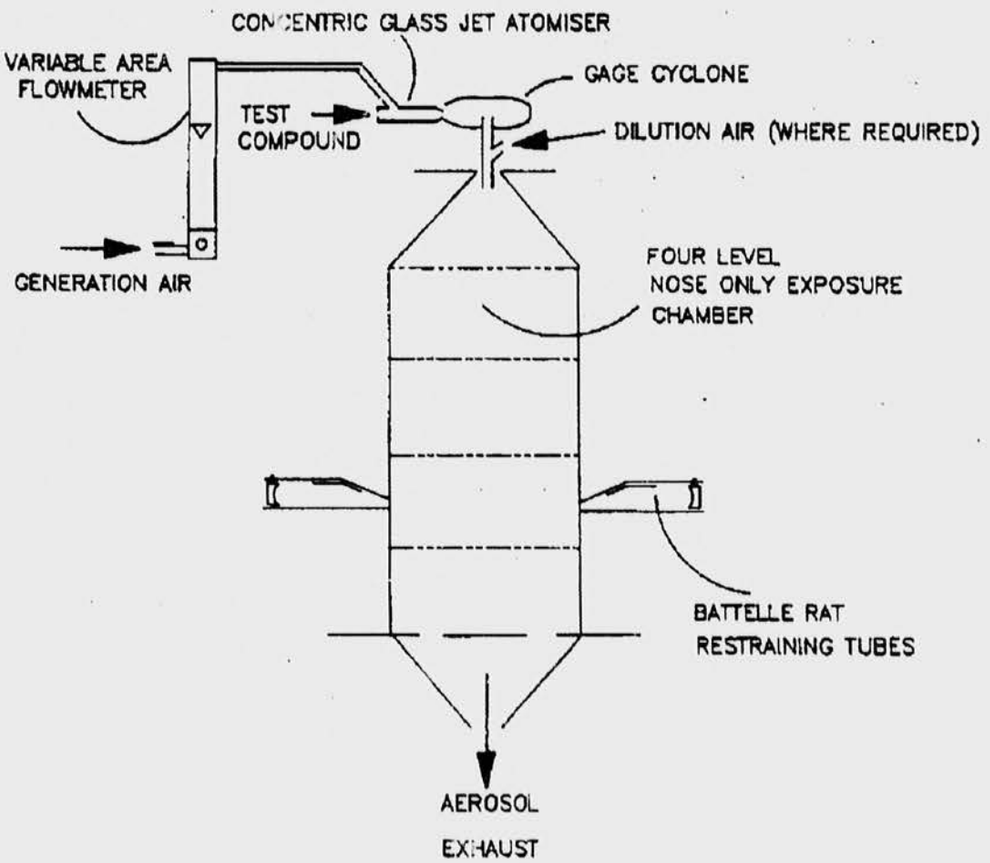
The restraining tubes were made by Battelle, Geneva and consisted of a polycarbonate tube, one end of which was tapered and fitted into the exposure chamber. In the other end a spring loaded plunger was fitted which was contoured to fit a rat and fitted over the base of the tail. The top of the tube had apertures to prevent excessive build-up of heat and water vapour which might make the animal unduly uncomfortable, while similarly there was a groove in the bottom of the tube for drainage of urine.

3. Sampling

Atmospheric concentrations were determined by sampling through the sampling port. While atmospheres were being set up prior to animal exposure the holes for the restraining tubes were plugged.

APPENDIX E - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

DIAGRAM OF 4–TIERED EXPOSURE CHAMBER
WITH CONCENTRIC JET GLASS ATOMISER
AND GAGE CYCLONE



APPENDIX F - EXPOSURE CHAMBER TEMPERATURES AND RELATIVE HUMIDITY

These data were taken from the readings of temperature and relative humidity recorded during the exposure period in the exposure chamber.

Group 1

Date	Temperature (°C)	Relative Humidity (%)
9/09/98	19.2-20.2	33-47
10/09/98	18.7-22.6	38-61
11/09/98	18.6-20.9	44-52
12/09/98	17.7-20.9	34-46
13/09/98	19.0-21.2	38-47
14/09/98	19.0-22.3	25-36
16/09/98	19.6-21.8	28-39
17/09/98	19.6-21.6	36-47
18/09/98	19.9-22.3	42-53
19/09/98	20.1-21.5	41-52
20/09/98	20.1-20.7	45-55
21/09/98	18.9-21.8	29-42
23/09/98	19.7-20.9	28-37
24/09/98	19.9-20.8	46-56
25/09/98	19.9-20.9	43-52
26/09/98	19.7-21.2	35-50
27/09/98	19.5-22.0	44-56
28/09/98	19.6-21.0	42-48
30/09/98	18.9-20.9	35-52
1/10/98	19.1-22.8	36-52
2/10/90	19.6-22.0	21-41
3/10/98	18.7-21.1	32-42
4/10/98	19.3-23.0	31-40
5/10/98	19.7-21.2	19-30

Group 2

Date	Temperature (°C)	Relative Humidity (%)
9/09/98	19.1-20.1	10-13
10/09/98	18.6-24.0	11-33
11/09/98	18.4-20.8	14-34
12/09/98	17.6-21.3	15-28
13/09/98	18.8-20.7	14-21
14/09/98	18.9-22.2	10-16
16/09/98	19.6-21.8	12-22
17/09/98	19.4-21.5	14-28
18/09/98	19.8-22.3	18-28
19/09/98	20.3-21.4	19-26
20/09/98	20.0-20.8	22-32
21/09/98	18.8-21.5	10-18
23/09/98	19.8-20.9	12-17
24/09/98	19.5-20.7	15-48
25/09/98	19.8-20.7	15-22
26/09/98	19.6-21.6	17-26
27/09/98	19.4-21.1	15-24
28/09/98	19.2-20.3	14-20
30/09/98	18.7-20.3	13-19
1/10/98	19.1-23.9	16-27
2/10/90	19.6-23.2	10-18
3/10/98	18.5-20.9	10-21
4/10/98	19.3-22.7	8-22
5/10/98	19.7-21.7	4-8

APPENDIX F - EXPOSURE CHAMBER TEMPERATURES AND RELATIVE HUMIDITY

These data were taken from the readings of temperature and relative humidity recorded during the exposure period in the exposure chamber.

Group 3

Date	Temperature (°C)	Relative Humidity (%)
9/09/98	19.1-20.1	13-19
10/09/98	18.6-23.4	15-32
11/09/98	18.4-20.9	14-31
12/09/98	17.6-21.1	16-39
13/09/98	18.9-20.9	17-25
14/09/98	18.9-22.1	11-15
16/09/98	19.6-21.7	14-22
17/09/98	19.4-21.5	15-27
18/09/98	19.8-22.0	21-28
19/09/98	20.7-21.3	23-30
20/09/98	20.0-20.6	22-35
21/09/98	18.9-21.4	11-22
23/09/98	19.8-20.8	15-20
24/09/98	19.8-20.9	19-26
25/09/98	19.8-21.1	15-27
26/09/98	19.7-21.8	17-28
27/09/98	19.5-21.6	16-27
28/09/98	19.5-20.9	15-23
30/09/98	18.7-20.5	14-20
1/10/98	19.1-23.7	15-26
2/10/90	19.6-22.8	13-20
3/10/98	18.6-20.9	14-22
4/10/98	19.4-22.6	9-17
5/10/98	19.8-21.5	4-9

Group 4

Date	Temperature (°C)	Relative Humidity (%)
9/09/98	19.1-20.4	9-16
10/09/98	18.6-20.7	26-47
11/09/98	18.6-20.1	23-38
12/09/98	17.7-20.9	24-37
13/09/98	19.0-20.9	21-34
14/09/98	18.8-21.6	10-16
16/09/98	19.5-21.7	10-17
17/09/98	19.5-21.4	18-28
18/09/98	19.9-22.1	23-38
19/09/98	20.4-21.4	20-29
20/09/98	20.0-20-7	18-35
21/09/98	19.0-21.3	10-20
23/09/98	19.9-20.8	9-14
24/09/98	19.8-21.1	20-27
25/09/98	19.9-21.0	18-31
26/09/98	19.7-21.7	19-28
27/09/98	19.5-21.1	23-35
28/09/98	19.6-20.9	12-21
30/09/98	18.7-20.5	12-18
1/10/98	19.2-23.5	21-35
2/10/90	19.6-23.1	14-24
3/10/98	18.6-20.9	19-24
4/10/98	20.0-22.5	11-22
5/10/98	19.9-21.5	6-11

APPENDIX G - DETAILS OF STATISTICAL METHODS

Bodyweights were considered by analysis of covariance on initial (day 1) bodyweight.

Lung weights were considered by analysis of variance and analysis of covariance on final bodyweight. Summary statistics are presented for lung to bodyweight ratios but these were not analysed statistically as the analysis of covariance provides a better method of allowing for differences in terminal bodyweights (Shirley, 1996).

BrdU labelling indices were considered by analysis of variance following a double arcsine transformation (Freeman and Tukey, 1950).

Lung lavage fluid data were considered by analysis of variance. Total cell counts, alveolar macrophage counts, polymorphonuclear leucocyte counts and lymphocyte/other counts were transformed before analysis using a natural logarithmic transformation.

All analyses for the main study and recovery phase were carried out separately.

Analyses of variance and covariance were carried out using the GLM procedure in SAS(1989). Analyses of bodyweight allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

The difference from control based on the analysis of bodyweight are also presented graphically in figure 3. The centre of each bar represents the mean percentage difference between control and treated group least-squares means and the top and bottom of each bar represent the upper and lower 95% confidence limits for this difference. A statistically significant difference between the treated group and the control group is present when the bar does not cross the

zero difference line. For ease of reference, lines have been added to the plots to show the difference of ± 10%.

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